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EVOLUTIONARY TRENDS IN THE FRUITING HEAD OF COMPOSITAE

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INTRODUCTION

While problems of origin and evolution of the flower have often been discussed in botanic literature, comparatively little attention has been devoted to fruit phylogeny. Our schemes of fruit classification are still based on gross morphological lines combined with certain features of dispersal biology and have changed little since Linné.

The difficulties met in phylogenetic treatment of fruits are twofold:

First is the great diversity of primary and derivative forms occurring in families characterized by their more or less uniform flowers, such as Cruciferae, Papilionaceae, Caryophyllaceae, and the like. In the Cruciferae, no less than nine main derivative fruit forms have been distinguished by the author (Zohary, 1948) and considerably more may be found in Papilionaceae (e.g. *Astragalus*, Zohary, 1939).

Second is the elaboration of the dispersal apparatus which may affect different fruit types in the same direction and similar fruit types in various directions. Alterations introduced in the basic fruit pattern by equipments and properties of dispersal may sometimes efface the original fruit structure entirely. For instance, fruits commonly classed as "berries" may have their origin in various kinds of capsules, legumes, schizocarps and fruit aggregates. The "samara" and the "nux" are no less heterogeneous in origin. Though evolution of the dispersal apparatus has its own trends it can hardly be separated from pure morphological evolution.

In spite of its complexity fruit phylogeny is a promising field of research very necessary to the taxonomist and morphologist. It may also widen our outlook on organic evolution by disclosing the specific tools with which nature operates on propagation organs.

In this paper I wish to deal with a small fragment of this comprehensive subject. By a review of certain biological features peculiar to the fruiting head of Compositae an attempt is made to outline a few discernible evolutionary trends affecting the fruit aggregate as a morphological-biological entity.

MONOSPERMOUS FRUITS

Taxonomists have long been aware of the fact that several natural groups show a trend towards reduction in the number of seeds per fruit culminating in monospermy. The derivative character of one-

seeded fruits, such as the achene, the caryopsis, the utricle, the nutlet, etc., is in the majority of cases easily traceable either by direct morphological evidence (such as the presence of rudimentary ovules, separation tissue of the pericarp, traces of septa, numerous carpels, etc.) or by indirect evidence from phylogeny.

Nucamentation, as this evolutionary trend may be termed, occurs in various families and fruits as can be seen from the following selected examples: in the Cruciferae the nutlet is obviously derived from a silique, a loment and a silicle (Zohary, 1948). In the Leguminosae the formation of the nutlet from a legume or a loment can easily be traced in *Astragalus*, *Trigonella*, *Ononis*, and *Onobrychis*. The one-seeded fruits of *Fumaria*, *Dipterygium*, and *Paronychia* are derived either from siliques or capsules, those of Labiatae (*Salvia dominica*, *Thymbra spicata*, *Teucrium polium*, etc.) and Umbelliferae (*Lagoecia*, certain species of Echinophoreae, etc.) originated from schizocarps. Since one-seeded fruits have a heterogeneous origin, it is not surprising that their classification has so far been inadequate.

In view of the fact that monospermy constitutes an ultimate stage in a certain trend in fruit evolution, the tracing of further evolutionary changes in the fruits in such taxonomic groups in which monospermy has become a fixed characteristic is bound to yield interesting results. Of the many families (Gramineae, Cyperaceae, Dipsaceae, Compositae, etc.), tribes and genera with one-seeded fruits, I have selected the Compositae, representing a large and highly advanced family likely to provide a deep insight into the subject.

Aggregate Fruits

A striking feature of most of the families and groups characterized by monospermy is that their one-seeded fruits are, or tend to become, aggregated in more or less distinct morphological units. The spikelets of Gramineae, Cyperaceae and of others, the heads of Dipsaceae and Compositae, the headlike cymes of certain Valerianaceae, are examples of such aggregates. In these instances the individual fruit is less subject to carpological evolution than the aggregate as a whole.

Students of pollination phenomena have often considered the highly organized flowering head of Compositae as the equivalent of a single "flower" (pseudanthium) (Troll, 1928). Similarly, from the biological and evolutionary point of view, the fruiting heads of this family may be regarded as single many-seeded fruits. Aggregation of fruits does not necessarily lead to improved dispersal in the conventional sense, as one may also doubt whether aggregation of the flowers always improves pollination (Good, 1931). Rather it often leads to new modes of dispersal, to the development of new kinds of disseminules, and to the complete loss of dispersal accessories. It is frequently associated with or followed by other evolutionary specializations affecting the structure of aggregate fruits and resulting in their further numerical reduction.

Structure of the Fruiting Head

The following discussion deals with some processes involved in converting the fruiting head into a structure resembling a capsule in appearance or in dispersal behavior. During the course of evolution in this family, the involucre of the head has been elaborated to such an extent that it now plays a very important part in dispersal. Originally a system of bracts with no obvious function, the involucre eventually became a container regulating, facilitating or impeding single-fruit dispersal. Of the characters indicating a high level of organization of the fruiting head the following may be mentioned.

(1) The involucre facilitates single-fruit dispersal by movements.

(a) Xerochasy. Periodical movements of the involucre are in most cases conditioned by diurnal changes of the moisture in the air. Accordingly, the involucre opens in dry and closes in moist condi-

tions, and mode, rate and time of dispersal of individual achenes are thus largely affected by these movements. In the majority of cases (*Senecio, Crepis* spp., etc.) these movements are governed by a cohesion mechanism and their duration is limited to a short period, whereas in other groups (e.g. *Onopordon*) the involucre leaves continue their movements long after dispersal has been affected. In the latter the movements are caused by a shrinkage mechanism (Steinbrinck, 1906; Zohary and Fahn, 1941). These Xerochastic characteristics of the involucre constitute, no doubt, an advanced stage in the evolution of the head, at least from a biological point of view.

(b) Hygrochasy. Hygrochasy, as seen in the opening of the involucre after being moistened, seems to be a much more advanced character than xerochasy. In nature hygrochastic heads do not release their achenes until the winter rains moisten the involucre leaves and cause them to diverge. Exposed to the impact of rain drops the achenes are detached and removed from the receptacle. Hygrochasy has been found in some species of *Asteriscus, Cichorium, Evax, Anvillea* and others. Here, too, the anatomical reasons are based on a shrinkage mechanism (Steinbrinck and Schinz, 1908; Zohary, 1937; Fahn, 1947).

Xerochastic and hygrochastic heads behave like many-seeded capsules, opening by means of valves.

(2) The involucre facilitates dispersal of aggregates.

In a number of species (*Centaurea squarrosa, C. virgata, C. leptocephala, C. aggregata, Cousinia ramosissima, C. aleppica, Jurinea Stachelinae* and many others) the mature head, whether enclosing many or few achenes, is dropped off as a whole without releasing the achenes before germination (fig. 1A).

Parallels of this feature, known as synaptospermy (Murbeck, 1920), are found in Liliaceae (*Bellevalia desertorum, Leopoldia eburnea*), Leguminosae (many species of *Astragalus, Medicago, Trigonella, Ornithopus,* etc.), Cruciferae (*Chorispora, Raphanus,* etc.), Labiatae (*Molucella laevis, Salvia* spp., etc.) and in other families.

In other species of Compositae synaptospermy is incomplete, as only the achenes near the involucre leaves remain attached to the head, which at last falls off as a whole either with or without the aid of an abscission layer in the upper part of the stalk (e.g. *Centaurea iberica, C. hyalolepis, C. procurrens, Catananche lutea, Carduus* spp., *Crepis aculeata, Reichardia tingitana* and many others (fig. 1B, C)). A similar behavior is found in simple fruits (e.g. *Sinapis* spp., *Eru-*

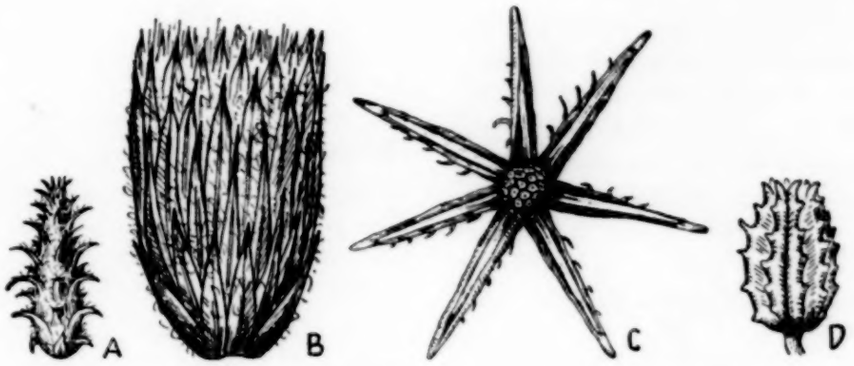

FIG. 1. A—*Centaurea virgata,* entire head as dispersal unit. B—*Carduus argentatus* and C—*Crepis aculeata,* involucre with marginal achenes as dispersal unit. D—*Crepis aspera,* involucre with marginal achenes not detached from the stem (dispersal impeded). (Twice natural size.)

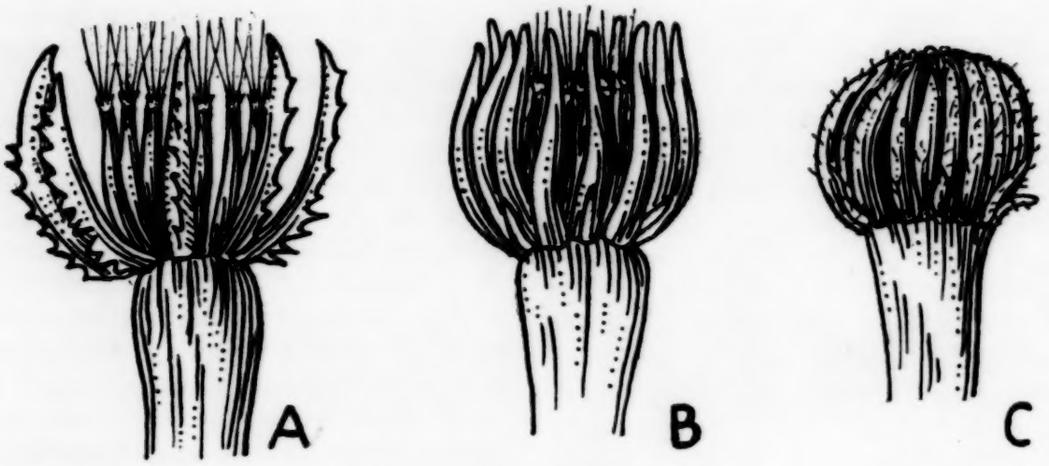

FIG. 2. Fruiting heads of three forms of *Hedypnois cretica*.
A—head opening after fruit ripening (full dispersal).
B—head half closed (dispersal of central achenes only).
C—entirely closed head (no dispersal of achenes). (Twice natural size.)

caria spp.) in which some of the seeds of the pod are dispersed singly and the others remain in the stylar part of the fruit.

(3) The involucre impedes dispersal. In certain species of Cichoriae, such as *Rhagadiolus stellatus, Garhadiolus* spp., certain forms of *Hedypnois cretica, Crepis aspera* (fig. 1D), *Hyoseris glabra,* some species of *Picris,* etc., the central achenes are dispersed singly, while the others are held in by the persistent and for the most part strongly lignified involucre leaves, without being removed either singly or as a group. Examination of these and certain forms of *Hedypnois cretica* discloses the possibility of establishing a continuous series leading to the stage in which all achenes of the fruiting head are kept closely together, attached to the receptacle and closed by the involucre leaves (*Hedypnois cretica forma clausa* (fig. 2C)). A condition analogous to such heads is found in the indehiscent capsules of certain species of *Scrophularia, Verbascum* and other genera.

DIFFERENTIATION IN AGGREGATE FRUITS

Heterocarpy is one of the most striking characters of the fruiting head of Compositae. In no other family is heterocarpy as well represented as here, notably among the Liguliflorae. Of the various kinds of heterocarpy two may be mentioned:

(a) Differences in size. A decrease in size of the achenes extends from the margin to the center of the head in many species of *Crepis, Picris, Launea, Rhagadiolus, Garhadiolus, Hyoseris, Anthemis, Calendula* and numerous other genera. Since in some cases (*Hyoseris, Launea,* etc.) the central achenes are often aborted, one could perhaps consider this centripetal decrease of the achenes as a trend to reduce the number of achenes through sterilization of the central florets. A similar feature is encountered in the Umbelliferae, where the inner fruits or achenes are either smaller in size (e.g. *Daucus, Torilis*) or one-seeded (e.g. *Ainsworthia Carmeli, Scandix palaestina,* and *Tordylium* spp.).

(b) Differences in pappus structure. The structure of the pappus varies considerably, as is evident from a comparison of the types listed below:

Catananche type: The pappus of the marginal achenes is shorter and poorer than that of the other achenes (*Catananche,* some species of *Crepis*).

Picris type: The pappus of the marginal achenes is reduced to a short crown-like

structure (species of *Picris, Thrincia, Leontodon, Seriola*).

Hedypnois type: Only a few central achenes are equipped with a normal chaffy pappus, while in all others the pappus is crown-like (certain forms of *Hedypnois, Hyoseris*).

Carthamus type: Marginal achenes without pappus, all others with normal pappus (most species of *Carthamus*, some species of *Centaurea* and others).

Anthemis type: The ear-shaped pappus decreases in size from the center to the margin (many species of *Anthemis, Chamaemelum, Aaronsohnia*).

Without raising the question of homology and origin of the pappus, one must agree with Small (1919) that the primary form of the fruit of the Compositae is that furnished with a well developed setose pappus as represented in *Senecio* and others. From this type all other pappus forms have been derived. Epapposity is thus in most cases a secondary phenomenon. In accordance with this view and the observation made in various genera in regard to differences in the structure and size of the pappus, the following conclusions may be drawn:

(1) An evolutionary trend toward reduction of the pappus in size may lead to its complete disappearance or to its turning into a crownlike structure. This trend can be traced in the genera of the Lapsaneae (fig. 3), as well as in *Carthamus, Anthemis*, and other genera.

(2) In all types mentioned above the reduction of the pappus has taken place in a centrifugal direction, although in most genera the transition from the central (with a normal pappus) to the marginal achenes (with no or a reduced pappus) is rather abrupt. The gradual reduction of the pappus in the genus *Hedypnois* (fig. 4) seems to justify this assumption.

(3) Reduction has taken place in various ways (abbreviation, depletion, deciduousness and total disappearance) and in various types of pappus (setose, plumose, paleaceous and auriculate).

(4) Although no casual explanation of the reductional process in the dispersal organs is available, it is clear that through the loss of the pappus or its partial reduction all or a part of the achenes of the head have become less fit for long-distance dispersal.

Heterocarpy is one of the clearest manifestations of differentiation among the members of an aggregate. The constant positions of the different members of an aggregate show that to a certain degree the members are interdependent. This condition provides additional proof of the view that carpological evolution in Compositae affects the aggregate as a whole rather than single fruits.

Reduction in the Number of Members in an Aggregate

Many genera and groups of genera show a marked tendency toward reducing the number of achenes per head to a minimum. This is mostly brought about through sterilization of most or part of the florets. In certain species this process

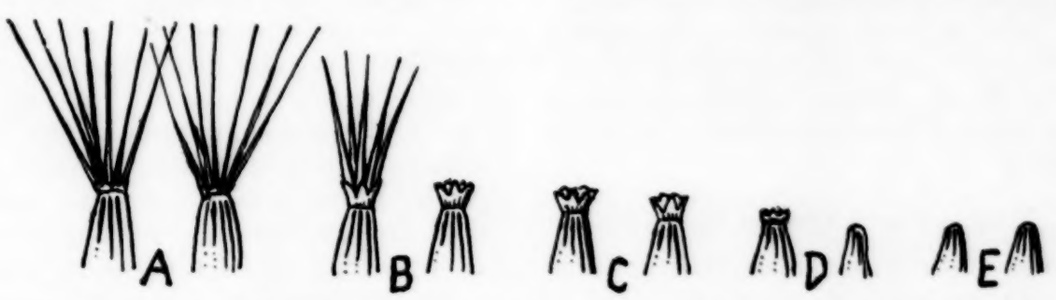

Fig. 3. Sketch showing the abortion of pappus within the Lapsaneae group; each pair represent a central (left) and a marginal (right) achene of the head. A—*Tolpis virgata*, B—*Garhadiolus papposus*, C—*Garrhadiolus Hedypnois*, C—*Rhagadiolus stellatus*, E—*Lapsana ramosissima* (achenes all sketchy).

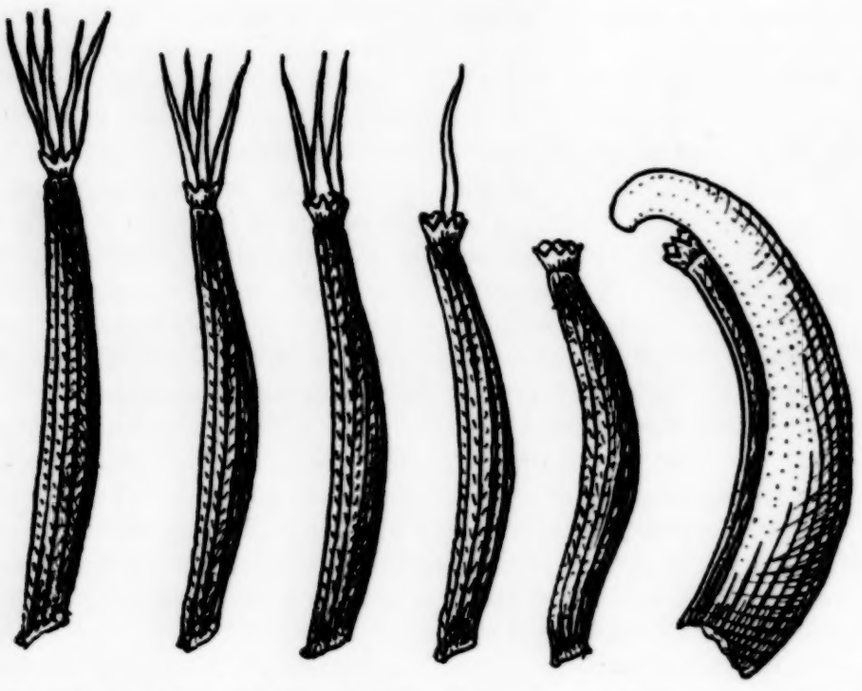

FIG. 4. Gradual reduction of pappus (inner row) from center towards margin of head in the closed form of *Hedypnois cretica*. (Eight times natural size.)

has gone so far as to turn the heads into one-seeded heads. Sterilization within the head took place both in a centripetal and a centrifugal direction. As examples of the former, mention may be made of the Calendulae, Anthemidae, Lapsanae, and Hyoseridinae. In *Artemisia monosperma* reduction has resulted in one-seeded heads. On the other hand the small one-seeded heads of the Gundeliae and Echinopsidae arose as a result of centrifugal sterilization. As in many genera of this and other families depletion of primary aggregates has been compensated by congestion of mono- or oligocarpous heads into secondary aggregates, such as are seen in *Echinops, Acantholepis, Evax, Filago,* several species of *Centaurea* (Sec. Acrolophus) and many others.

PARALLELS IN OTHER FAMILIES

The evolutionary processes mentioned above are characteristic also of other families and genera in which numerical reduction has led to one- or few-seeded fruits. In some instances certain stages may well have been less complete or even omitted. In the Gramineae, the many-flowered spikelets of the grasses constitute a well organized primary aggregate. Differentiation occurs in heterocarpous spikelets (e.g., in the Aveneae, Triticae, etc.), in which synaptospermy is also abundant. Numerical reduction of the fruits within the aggregate through sterilization is very common in various tribes. In some genera (*Phalaris paradoxa, Aegilops longissima,* etc.) spikes or headlike panicles comprise heteromorphous spikelets. Reduction through sterilization of entire spikelets as in *Aegilops, Cynosurus, Vulpia* and many others often results in the formation of higher dispersal units, such as entire spikes or heads.

In Umbelliferae reduction has not reached the stage of one-seeded fruits, except in a few genera. Yet the family shows a marked trend of fruiting umbellets or umbels functioning as organized carpological aggregates. In addition xerochasy (e.g. *Daucus*), hygrochasy (*Ammi, Visnaga*), synaptospermy (*Anisosciadium, Echinosciadium*), heterocarpy (Daucinae,

Tordylium, Ainsworthia) and numerical reduction of fruits per umbellets (in certain Echinophoreae) are known.

Similar trends are traceable in the Paronychiae (e.g. *Paronychia, Scleroccphalus, Pteranthus, Gymnocarpus, Cometes*) where numerical reduction of seeds per fruit has led to the formation of aggregates.

Summary and Conclusions

There is a general trend in fruit evolution of certain groups towards reduction of the number of seeds per fruit resulting in the formation on one-seeded nutlets. The latter become often aggregated into organized units which undergo further developmental processes both in form and in dispersal behavior. Evolution, however, affects the aggregate as a whole rather than the individual fruits. At a more advanced stage the number of fruits per aggregate tends to be reduced and in turn such depleted aggregates are reorganized into secondary aggregates.

In Compositae aggregation of one-seeded fruits has resulted in the formation of a well-defined carpological unit imitating a capsule or a silique in its dispersal behavior and structural peculiarities of the involucre. Differentiation within the aggregate as manifested in heterocarpy of various kinds is closely connected with differentiation in dispersal. Partial sterilization of the members of the aggregate may be considered as a secondary trend towards mono- or oligospermy of the heads leading to or associated with secondary aggregation.

The above consideration may broaden our view on carpological evolution far beyond the limits of conventional morphology. They may also lead us to regard the fruiting head as a biological subject *per se*, more complex in its evolutionary problems than the flowering head.

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POLLINATION RELATIONS OF *LYCOPERSICON ESCULENTUM* IN NATIVE AND FOREIGN REGIONS

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INTRODUCTION

Many of our important cultivated plants are cross-pollinated in their native habitat; when grown in other areas their pollination relations may present problems of general biological and practical significance. An unstable condition in foreign areas is anticipated since the flowers may be visited by alien pollen vectors or they may not be visited at all. Reproduction may be seriously affected in an obligatorily cross-pollinated plant—for example, alfalfa (*Medicago sativa* L.) growing in certain areas in California, where seed production seems to be limited by the fact that the flowers are not always visited by insect species that can adequately cross-pollinate them (Linsley, 1946).

Among plants commonly accepted to be completely self-pollinated, the garden pea (*Pisum sativum* L.) was recently found to be cross-pollinated to the extent of 3.3 per cent in Perú, a region that is outside the native habitat of this species (Harland, 1948). Again, differences as great as tenfold in rates of natural cross-pollination of the cultivated tomato (*Lycopersicon esculentum* Mill.) were found in various localities in California (Rick, 1949). Such variations obviously have great bearing on breeding practices, methods of seed production, and even yields of the agricultural commodities themselves.

Surprising variations are encountered even among anemophilous plants—for example, common wheat (*Triticum vulgare* Vill.), which is usually assumed to be highly self-pollinated. Rates of natural cross-pollination as high as 34 per cent were found in the United States by Leighty and Taylor (1927), and a large number of natural hybrids between cultivated varieties were recognized by Howard *et al.* (1910) in the Punjab, where they observed that in the dry climate the glumes often opened, thereby exposing the stigmas to wind-borne pollen.

The intrinsic pollination relations of a species are often assumed to be the same as those observed under cultivation in foreign areas. The examples of wheat and tomato just cited show how misleading such assumptions can be. The pollination relations, especially of facultatively cross-pollinated plants, in their native habitat can scarcely be predicted from information gained in foreign regions. Although the former relations may have important practical and theoretical bearing, extremely little is known about them.

The tomato has been regarded as a highly self-pollinated plant, and until recently all published tests have indicated very low rates of natural cross-pollination in eight stations in the North Temperate Zone, all outside the range of distribution of the genus *Lycopersicon* (literature reviewed by Rick, 1949). On the other hand, two recently discovered facts hint that rates might be higher in the indigenous region; namely, rates vary greatly within and between localities in California; and *L. peruvianum* (L.) Mill.* and related species were found to be genetically self-incompatible and consequently dependent upon pollen vectors for their reproduction in nature (McGuire, unpublished). A study was therefore undertaken of the pollination mechanisms of wild and cultivated tomato species in the Andean region where the genus is native. The present report deals only with the study of *L. esculentum* and a

* Specific names are used here according to Muller's treatment (1940).

comparison with observations in non-native areas, particularly California.

Materials and Methods

Since one of the purposes of this study was to compare rates of natural cross-pollination (hereafter abbreviated NCP) in the Andean region with those previously obtained in California, it was imperative to utilize the same materials and to adhere as closely as possible to the methods that had been employed in the three-year tests in the latter region (Rick, 1949).

Genetically male-sterile tomatoes, which are mutants that do not produce viable pollen but have normal ovule fertility, can be used to advantage in testing NCP. All fruits and seeds that they yield must be generated by pollen that is transferred to them from fertile plants.

In the California tests, only two mutants—ms_3 and ms_9, which have anthers of nearly normal form and color—had been used. It had been discovered previously (Rick, 1948) that of the tested male-sterile mutants, these enjoy the highest rates of NCP presumably because pollen vectors prefer to visit flowers with the most nearly normal anthers. Accordingly ms_3 and ms_9 were used in the new plantings. The mutant ms_{10}, which displays the greatest degree of anther abnormality and lowest rates of NCP in California, was also included in certain plots for purposes of comparison. All mutants used belong to the variety San Marzano.

Following methods previously used, male-sterile plants that had been identified in segregating backcrosses in the nursery were alternated with fertile plants in a checkerboard fashion in the field planting. Plants were spaced 4½ to 5 ft. apart in rows spaced similar distances. Four plants each of the three mutants were placed in random positions in the plantings.

The following considerations governed the location of plots. It was felt necessary that each plot be placed within the range of native species of *Lycopersicon*—that is, a narrow strip of land extending inland from the coast to about 3,000 m. altitude and reaching from northern Chile to central Ecuador. The valley of the Rimac River was chosen for several of the plots because it lies in the center of this distribution and is accessible for the frequent visits that are required by test plots of this type. Other plots were planted at Arequipa, Cañete, and Tingo Maria, all within Perú, but their usefulness was restricted for reasons subsequently revealed.

Three sites that were chosen within the valley of the Rimac were: (1) the Centro Nacional de Investigación y Experimentación Agricola de La Molina at 250 m. elevation and about 8 km. from the coast, (2) the experimental grounds of the Sociedad Nacional Agraria at Ñaña at about 600 m. elevation and some 26 km. from the coast, and (3) a fruit-tree nursery of the Ministerio de Agricultura at Santa Eulalia at some 1,200 m. elevation and 55 km. inland. Although *L. esculentum* var. *cerasiforme* (Dun.) Gray is known in the valley, it is not abundant. The very closely related *L. pimpinellifolium* (Jusl.) Mill. is ubiquitous and occurs near all three sites. La Molina lies within the range of *L. peruvianum* (L.) Mill., Santa Eulalia at the lower edge of the range of *L. glandulosum* C. H. Mul., and Ñaña in the range of forms that are intermediate between the two latter species. No suitable arrangement could be made to plant a test plot within the range of *L. hirsutum* (Humb. and Bonpl.) (2,000 to 3,000 m.).

The mutants ms_3, ms_9, and ms_{10} were planted at La Molina and Ñaña. All other plots lacked the ms_{10} mutant.

All plots were planted in November and December, 1948. Because the plants grew rapidly and matured early, the flowering period was largely confined to January, February, and early March, 1949. This period represents the end of the season of greatest flowering and vector activity of *L. peruvianum* and the beginning of that of *L. glandulosum*. The

TABLE 1. *Yields of seeds and fruits of fertile and male-sterile plants in test plots in Perú*

Station	Mutant	Seeds per fruit		Per cent flowers that set fruit		Seeds per flower*		Seeds per plant
		No.	Per cent St/Ft**	No.	Per cent St/Ft**	No.	Per cent St/Ft**	
La Molina	Fertile	50.2		55.1		27.7		6,190
	ms_3	5.9	11.7	4.2	7.6	0.25	0.9	62.8
	ms_9	12.7	25.3	12.4	22.5	1.57	5.7	224
	ms_{10}	33.3	66.3	42.7	77.5	14.2	51.4	2,567
Ñaña	Fertile	51.3		70.3		36.1		6,720
	ms_3	6.0	11.7	0.6	0.9	0.036	0.1	9.6
	ms_9	15.9	31.0	10.0	14.2	1.59	4.4	332
	ms_{10}	36.4	70.9	40.7	57.9	14.8	41.1	2,861
Santa Eulalia	Fertile	68.6		50.1		34.4		4,900
	ms_3	2.9	4.2	1.2	2.4	0.035	0.1	15.3
	ms_9	12.4	18.1	9.6	19.2	1.19	3.5	498

* Product of seeds per fruit and per cent flowers that set fruit.
** Per cent St/Ft is the sterile-plant yield expressed as a percentage of the fertile-plant yield.

flowering of *L. pimpinellifolium* and *L. esculentum* culminates in this period, although wherever irrigated they will flower and fruit throughout the year. It is the warmest part of the year for this region and the season to which cultivated tomatoes are best adapted.

The methods of measurement were based on experience gained in preceding studies. The percentage of flowers that set fruit was determined by examining pedicels of all inflorescences of sterile and fertile plants at the end of the season. The number of seeds per fruit was counted in a representative sample of 20 fruits (fewer in less reproductive plants). It was necessary to derive total fruit number by counting pedicels because fruits often dropped prematurely and decayed rapidly after severe attack by worms. Total seed production calculated as the product of this number and the estimated mean number of seeds per fruit is likely subject to considerable error and the values thereby obtained should be interpreted accordingly.

OBSERVATIONS OF THE TEST PLOTS

All observations are summarized in table 1. Yields of sterile and fertile plants are given for seeds per fruit, per cent flowers that set fruit, seeds per flower, and the total number of seeds per plant, the latter being calculated in the manner previously described. The number of seeds per flower—the product of the mean number of seeds per fruit and the per cent flowers that set fruit—is a convenient and probably the most reliable single measure of NCP (Rick, 1949). Since growing conditions can vary greatly from one plot to another, it seems possible that the fruit-setting of sterile plants might be limited by certain factors that limit yields of fertile plants in the same plot. For this reason sterile-plant yields are also expressed in table 1 as a percentage of the fertile-plant yield when the latter is arbitrarily considered as 100 per cent.

Before values for Perú can be compared with those of California, the enormous differences in rates of NCP between ms_{10} and the other mutants must be noted and properly interpreted. In terms of number of seeds per fruit, the yield of ms_{10} is more than twice that of ms_9 and more than five times that of ms_3 in both the La Molina and Ñaña plots. In per cent flowers that set fruit the difference is even greater, the value for ms_{10} being more than three

times that of ms_9 and ten times that of ms_3. This result is completely contradictory to the previous report (Rick, 1948) based on tests for two years at Davis, California, in which it had been found that the fruit-setting fraction of flowers of ms_{10} *was less than 30 per cent of that of* ms_9. Plants of ms_3 had not been included in the latter study, but their rates in other testings have been approximately the same as those for ms_9. To aid in understanding the contrast between the fruit setting behavior of ms_9 and ms_{10} in Perú and California, the respective values are presented graphically in figure 1.

What could account for this wholly unanticipated reversal of rates? In the California experience the strong positive correlation between size of anther and rate of NCP in nine different mutants studied suggested that the difference in rates depends upon attractiveness of the anther tube to the insect pollen vectors. It seems unlikely that the vectors in Perú have tastes of an opposite nature to those in California; furthermore, it was observed in Perú that the responsible pollen vectors were active to more or less the same extent around flowers of all the mutants used.

Examination of the anther tubes provided a reasonable explanation of this reversal in rates of NCP. In examining flowers of all plants in the field in order to confirm the positions of fertile and sterile plants, it was noticed that stigmas of ms_3 and ms_9 were depressed below the tip of the anther tube, contrary to the position at the same level as the tip observed in the same stocks grown in California. The stigmas of ms_{10} are always exserted in the Peruvian as in the Californian plots, thanks to the great foreshortening of the anthers in this mutant. These relations were found consistently in observations of all Peruvian plots at various times during the flowering season. Representative positions of the stigmas are given in table 2.

The stigmas of ms_3 and ms_9 in Perú apparently are seldom accessible enough for the visiting insects to deliver pollen to them. The depression of the stigmas is not great, but it is quite consistent; furthermore, the orifice of the anther tube of the San Marzano variety is very small, rendering the delivery of pollen still more difficult. While it is not proved absolutely that the great reduction in rates of NCP is caused by this slight change in flower form, it appears the more likely because the greater depression of stigmas of ms_3 (table 2) is consistently related with its much lower level of NCP at all stations (table 1).

Position of the stigma as a factor affecting NCP relations in the tomato has been recognized previously by Lesley (1924), who discovered much higher rates in the variety Magnus having stigmas exserted

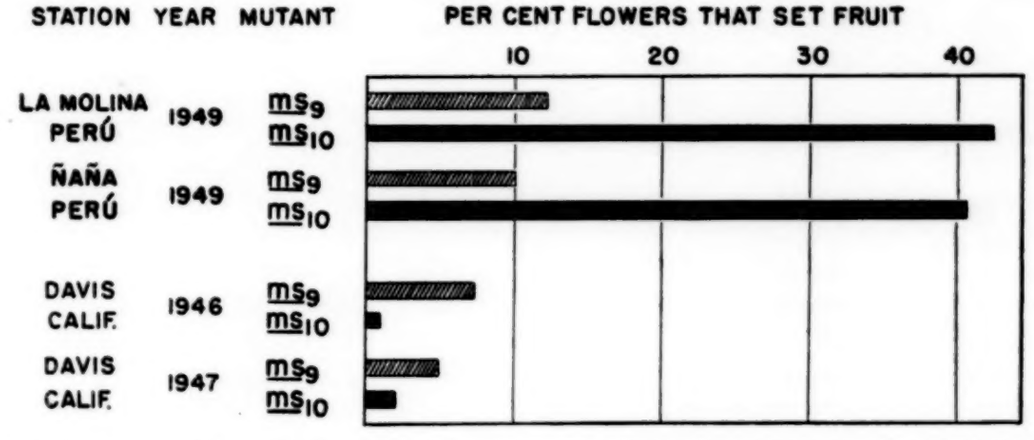

FIG. 1. Comparative rates of natural cross-pollination of ms_9 and ms_{10} in Perú and California.

TABLE 2. *Distance from tip of stigma to tip of anther tube**
(Measurements in mm.)

Station	Fertile	Mutant		
		ms_3	ms_9	ms_{10}
La Molina	−0.31 ± 0.11**	−1.12 ± 0.09**	−0.59 ± 0.10**	
Ñaña	+0.25 ± 0.08	−1.20 ± 0.09	−0.52 ± 0.04	+0.88 ± 0.14**
Santa Eulalia	−0.03 ± 0.08	−0.88 ± 0.07	−0.48 ± 0.03	
Davis, California	−0.14 ± 0.04		−0.11 ± 0.04	+1.0 ± 0.3

* A positive value signifies an exserted stigma; a negative value signifies a stigma depressed within the anther tube.
** Standard error.

1.4 mm. than in the variety Dwarf Champion, which has stigmas depressed 1.0 mm. below the anther tips.

One can only speculate as to the environmental factor responsible for this difference in flower form between plants cultivated in California and those in Perú. Mean temperatures are so similar that they can scarcely be suspected. Diurnal fluctuations in temperature on the other hand are notably greater in most of the areas of tomato production in California than in Perú. The climates also differ in length of photoperiod and in relative humidity. Howlett (1939) found differences in stigma position associated with changes in day length, light intensity, and nitrogen-carbohydrate balance, but the causative relationship was not clear. Whatever the responsible factors, another curious example must be added to the list of slight morphological changes that can profoundly affect the pollination relations of the tomato flower (Rick, 1947 and 1948).

Previous work in which mutants ms_3 and ms_9 have been used to measure NCP (Rick, 1949) should be reconsidered in the light of the discovery of this variable stigma position. It is conceivable *a priori* that the tenfold differences in rates observed in different localities in California might represent variations in suppression of the stigma and not variations in activity of the pollinating insects. This argument cannot be refuted completely since the stigma position was not examined throughout the season in all localities tested. It is certain, nevertheless, that stigmas were well exposed in the seasons at Davis when very low levels of NCP were recorded. The stigma position for ms_9, ms_{10}, and fertile plants grown at Davis in 1949 is given in table 2. Stigma position likewise cannot account for the large differences between 9 different mutants observed in a two-year period at Davis (Rick, 1948).

For the reason just presented comparisons of rates of NCP of mutants ms_3 and ms_9 in Perú and California would not be valid. Attention must be directed instead to ms_{10}, for which mutant observations in California are unfortunately limited to testings of the percentage of flowers that set fruit in two seasons at Davis. Observations in Perú are similarly restricted only to the plots at La Molina and Ñaña because only in these was ms_{10} planted. The data are admittedly fragmentary and not as ample from either California or Perú as had been hoped, but the results are so consistent and conform so well with correlative observations that they are deemed worth reporting.

The only exact comparison that can be drawn is between the per cent flowers that set fruit in the 1946 and 1947 data from

Davis, California and the 1949 data from La Molina and Ñaña, Perú. The values for California are respectively 1.3 and 2.1 per cent, while their counterparts in Perú are 42.7 and 40.7 per cent (see fig. 1).

In order to compare the two areas on the basis of other measures, Peruvian figures for ms_{10} must be matched with those for ms_9 in California. Such comparisons presumably put the Peruvian data at a disadvantage because ms_{10} is known to have much lower rates of NCP in California than ms_9. The observations in Perú might be further handicapped by the fact that, for practical purposes, localities of highest rates were sought in placing the test plots in California, where the variations in rates between localities are therefore probably greater, and the highest rates observed probably much higher, than those that would have been obtained from random sampling. The comparisons are instructive, nevertheless, since the Peruvian figures are consistently higher. For these comparisons the mean number of seeds per flower, probably the best single measure of NCP, is used as the basis of comparison. Values for the California data (Rick, 1949) were calculated and are presented in table 3. The highest rates of NCP, which were observed at Capay, a station very close to nesting sites of the pollinators, are 5.16 and 7.02 seeds per flower, being respectively 32 and 33 per cent of the yields of fertile plants. The other rates for California plots vary from 2.54 to 0.32 seeds per fruit, or from 19 down to 1.0 per cent, the majority of the values being below eight per cent of the fertile-plant yield. The sterile-plant values for La Molina and Ñaña are respectively 14.2 and 14.8 seeds per flower, being 51.4 and 41.1 per cent of the fertile-plant rates. The rates measured in these two localities in Perú are therefore higher than the highest values in California and are very much higher than the general level of rates measured in California and elsewhere in the North Temperate Zone.

TABLE 3. *Seeds per flower (product of seeds per fruit and per cent flowers that set fruit) for various localities in California (calculated from Rick, 1949)*

Plot No.	Station	Sterile	Fertile	Per cent St/Ft*
451	Santa Paula	0.61	63.5	1.0
452	Westminster	0.49	20.5	2.4
453	Riverside	0.41	27.0	1.5
454	Bonita	0.32	20.2	1.6
455	Davis	1.59	8.3	19.
456	Capay	5.16	16.0	32.
461	Capay	1.38	17.6	7.8
462	Capay	2.54	19.8	13.
463	Davis	1.56	19.5	8.0
471	Capay	7.02	21.0	33.
472	Riverside	0.56	9.3	6.0
473	Bonita	0.93	43.4	2.1
474	Oceanside	2.05	49.2	4.2
475	Davis	0.57	23.4	2.4
476	Davis	0.61	19.9	3.1
477	Davis	0.54	17.4	3.1

* Per cent St/Ft is the sterile-plant yield expressed as a percentage of the fertile-plant yield.

Rates of NCP of the ms_8 and ms_9 mutants, which are comparable with those of the La Molina and Ñaña plots, were observed in the additional plots at Cañete, Moquegua, and Santa Eulalia (the plots at Arequipa and Tingo Maria failed), but these have doubtful significance because the submerged stigmas of these mutants apparently prevent them from satisfactorily assaying full rates of NCP.

Another aspect of these data is the gradual reduction in rates of NCP from La Molina at the lower end of the Rimac Valley to Santa Eulalia, the most interior station tested. If the products for sterile plants expressed as percentages of the fertile-plant yields are examined in table 1, it will be seen that the values for each mutant decline consistently from La Molina to Ñaña and from Ñaña to Santa Eulalia. The consistency of this trend makes it seem significant, but one can only speculate as to its meaning. It might be related to the previously mentioned differences in distribution of native

species of *Lycopersicon*, the differences in their season of flowering, and consequent effect on rates of vector activity.

The reduced interplant and interbranch variation of the Peruvian data also deserves mention. As an example, the coefficient of variation was calculated for per cent flowers of ms_{10} that set fruit in the plots at La Molina and Ñaña. The validity of such statistics is open to question because they are calculated from ratios of two variables and from a very small number of variates. Certainly any attempt to demonstrate statistical significance would not be justified. The values are presented only because they agree with the impressions that were gained from the frequent casual inspections of the test plots. Coefficients for fertile plants at La Molina and Ñaña are both 12 per cent and for ms_{10} at these respective stations, five and 25 per cent. Corresponding coefficients for male-sterile plants in California tests vary from 15 to 50 per cent (only two plots in 12 have values as low as 25 per cent), and are never lower than those for fertile plants. The consistently high variability in the California data is construed to represent selection by the vectors of certain branches and plants in favor of others. Following this interpretation, reduced variability might be expected in the Peruvian data because with the greatly increased number of visits, the pollinators would compete more for flowers, and their visits would become more equally distributed.

For reasons pointed out previously (Rick, 1949) rates of NCP measured by the use of male-sterile plants do not necessarily apply directly to rates in fertile plants. Since the flowers of male-sterile plants produce no pollen to compete with that carried to them from fertile plants, it is suspected that unnaturally high rates would thereby be recorded; nevertheless preliminary unpublished tests at Davis indicate that rates of almost equal magnitude are obtained when fertile plants are tested by means of the proportion of hybrid seedlings in their progeny. At any rate, the much higher rates in Perú measured with male-sterile plants undoubtedly signify higher rates in fertile plants as well. Other observations that support this contention are presented in the following section.

Additional Observations

The tests just presented served to measure NCP in a limited region for a limited period of time; evidence of NCP in other areas and at different times is desirable. The following correlative observations are therefore presented to satisfy in part this need.

Another fact that testifies to the high rate of NCP in cultivated tomatoes in Perú is the frequent appearance of natural hybrids in field plantings. *L. pimpinellifolium*, which occurs in the areas of commercial tomato production, differs so greatly from cultivated varieties of *L. esculentum* in size of organs, in hairiness, and other characters, that the F_1 hybrids, which have often been produced artificially and have been studied by many investigators, can be distinguished from either parent without difficulty. The writer had the opportunity to count the proportion of F_1 hybrids in seedbeds of varieties of *L. esculentum* at La Molina. For this and many other privileges the writer is greatly indebted to Dr. V. L. Guzmán, Chief of the Department of Horticulture. The hybrid seedlings were readily distinguished by the aforementioned characters and also by their complete resistance to a species of *Oidium*, a pathogen that had severely attacked all the plants of *L. esculentum* in these seedbeds. Of a total of 706 seedlings, 25 or 3.5 per cent were hybrids. This is a comparatively low percentage when considered by itself, but it seems especially high in consideration of word from Dr. Guzmán that the *L. esculentum* parent plants had been grown at least 100 ft. from the nearest plants of *L. pimpinellifolium*. Previous studies have shown that natural crossing at such distances is quite exceptional. The writer was also informed

that these hybrids may occur at times in much higher frequencies. Intervarietal hybrids of *L. esculentum* also appear frequently and seriously contaminate tomato stocks according to information from tomato growers in the vicinity of Lima and Cañete.

Hybrids of less certain origin have also observed. A group of plants collected by the writer in an abandoned field near Pacasmayo is undoubtedly a hybrid swarm. Characteristics of *L. pimpinellifolium* predominate, although features of another species, probably *L. esculentum* appear in nearly all plants.

Observations of pollen-collecting bees frequently visiting tomato flowers are commonplace in Perú. The vectors are readily collected in large numbers in short periods of time from cultivated tomatoes. At La Molina, for instance, these insects were collected on several occasions at the rate of about one per minute. Attempts to find vectors in field tomatoes at Davis, California have been much less successful, the highest rate of collecting being on the order of one every 20 or 30 minutes. The rate of flower visiting in Perú is so intense that flowers of fertile plants are often completely divested of their pollen, which, when desired for use in controlled crosses, may often be difficult to obtain.

The activity of pollinating bees in cultivated tomatoes in Perú is matched by that in the wild species, for which similar observations were made. This situation is to be expected because certain of the latter, by virtue of their self-incompatibility, depend entirely on pollen transfer for their reproduction and survival.

As part of this project the insects pollinating tomato flowers were collected whenever possible. The great activity of the collected bees in vibrating the flowers with rapid leg movements accompanied by a high pitched hum leaves no doubt that they were collecting pollen and were responsible for cross-pollination. Furthermore, the contents of the pollen baskets of 12 of the insects representing most of the species of both host and vector were examined microscopically, and pollen with the characteristics of tomato species was identified in each case. Other insects that were occasionally found alighted passively on the flowers were not collected. The identifications and other relevant information is presented in table 4. The writer gratefully acknowledges the identification of the specimens by Mr. P. H. Timberlake of the Citrus Experiment Station, University of California at Riverside.

According to table 4 the bees pollinating the various tomato species are highly diversified: the collection includes eleven species representing six widely differing families. As verified by six different collections and casual observations on an equal number of occasions, chiefly at La Molina and Ñaña, the very closely related *L. esculentum* and *L. pimpinellifolium* are pollinated mostly by *Augochlora nigromarginata* and to a lesser extent by *Exomalopsis bruesi*. Even more interesting is the fact that these vectors were collected only once from any of the other tomato species, and on that occasion only a single specimen of each was found. The collections are too limited to establish whether each species of *Lycopersicon* has its own distinct group of pollen vectors. According to the general behavior patterns in certain of the families represented in table 4, their members might not be expected to remain constant to one species of flowering plant; moreover, the pollen grains present on several of the vectors betray that they had also been visiting other distantly related plant species. Whatever the pollen vector relationships of all the tomato species, the data suggest that the vectors of *L. esculentum* and *L. pimpinellifolium* are relatively distinct from those of the other species. Such specialization would seemingly follow a considerable evolution and suggest that the activity of the vectors is of fundamental importance, not only to the self-incompatible species, for whose

TABLE 4. *Collection data concerning species of bees that were taken while visiting flowers of* Lycopersicon *species in Perú (identifications by P. H. Timberlake)*

Host species	Site of collection*	Approximate elevation (meters)	Date of collection	Number of specimens										Remarks
				Colletidae	Halictidae		Anthophoridae				Xylocopidae		Euglossidae	Bombidae	
				Lonchopria (*Biglossidia*) sp.	*Augochlora nigromarginata* Spinola	*Augochlora matucanensis* Cockerell	*Anthophora tricincta* Friese	*Anthophora arequipensis* Brethes	*Exomalopsis bruesi* Cockerell	*Thygater albiceps* (Friese)	*Xylocopa brasilianorum* (L.)	*Xylocopa* sp.	*Centris surinamensis* (L.)	*Bombus funebris* Smith	
L. esculentum	Cañete	50	Dec. 30, 1948		1										At least 25 more of same species seen.
L. esculentum	Ñaña	600	Dec. 30, 1948		6				2						*A. nigromarginata* is much more abundant than *E. bruesi*.
L. esculentum	Pueblo Nuevo	100	Jan. 12, 1949		1										Four more of same species seen.
L. esculentum	Ñaña	600	Feb. 2, 1949		5										Many more of same species but none of *E. bruesi* seen.
L. esculentum	La Molina	250	Feb. 4, 1949		5										At least 10 more of same species seen.
L. esculentum	Santa Eulalia	1,200	Feb. 10, 1949		2										A few more of same species seen.
L. esculentum	La Molina	250	Feb. 25, 1949		1										Many more of same species seen.
L. pimpinellifolium	La Molina	250	Feb. 25, 1949		2										Many more of same species seen.
L. glandulosum	Canta	3,000	Feb. 8, 1949											4	Three more of same species seen; another sp. possibly *Anthophora* escaped.
L. glandulosum	Surco	2,000	Feb. 13, 1949			1				1					No other specimens seen.
L. hirsutum	Obrajilla	3,000	Feb. 8, 1949									1			No other specimens seen in two-hour period.
L. hirsutum	Surco	2,000	April 19, 1949										1		Two more of same species seen.
L. hirsutum	Surco	2,000	April 27, 1949	1											An entirely different species seen but not collected.
L. peruvianum	San Jerónimo	600	Dec. 8, 1948				1								Several more of same species seen.
L. peruvianum	San Jerónimo	600	Jan. 1, 1949				2	1							Many more of *A. tricincta* or possibly both species seen.
L. peruvianum	San Juan	50	Jan. 8, 1949		1				1		1				No other specimens seen.

* Pueblo Nuevo is in the Department of Libertad; all other stations are in the Department of Lima.

** Remarks are notes made in the field by the writer; identification is based on habits and appearance at short distances, in which characteristics these species differ greatly.

reproduction it is essential, but to the self-compatible ones as well.

It is of interest to note that the most active vector of tomato pollen in California, *Anthophora urbana* Cressm., belongs to the same genus as two of the very active vectors of pollen in Perú.

All of the evidence based on actual tests of rates of NCP, appearance of natural hybrids, and direct observations of vector activity therefore points to high rates of NCP of the cultivated as well as the wild species of *Lycopersicon* in Perú.

Discussion

The possibility of producing commercial F_1 hybrid tomato seed in Perú is suggested by this study. By interplanting fertile plants of one parent with male-sterile mutants of the other parent, substantial yields of hybrid seed could be obtained without the need of hand labor for either emasculation or pollination. Further testing would probably reveal other localities and seasons with even higher rates of NCP. At the present time, however, several factors obstruct the exploitation of these high rates. The foremost of these is the great assortment of pests, the control of some of which is still not properly understood. The insect problem is more serious in the proposed method of producing hybrid seed than in the production of fruit for market because the very measures used to control the harmful insects might discourage the pollinating insects. This problem can probably be solved; in this respect it was noted on several occasions that applications of DDT as a spray or dust controlled the noxious insects and yet discouraged the pollen vectors for a period of only about two days following the application. Nematodes and certain foliage diseases constitute other obstacles to tomato production in some seasons. Adaptability of varieties is another important consideration.

Another practical bearing of these findings relates to the practices of plant breeders and seedsmen. In most areas of cultivation the rates of NCP are so low that the tomato can be treated safely as a completely self-pollinated crop. Obviously in the Andean Region and possibly in other areas, however, the vectors are so active that the breeder must take special precautions to avoid contamination in controlled pollinations. For the same reason seed production plots will require spatial isolation.

It is also interesting to consider some of the implications of these observations in the evolution of the cultivated tomato.

According to the present evidence *L. esculentum* is subject to high rates of NCP in coastal Perú, in which region it is native, whereas it is nearly always self-pollinated in those regions of the world where it occurs only as a cultigen and where rates of NCP have been measured. This change of pollination relations raises important questions. First, what, if any, changes in flower form accompanied domestication and culture outside the natural range of distribution? Successful varieties cultivated in the latter regions have flowers whose stigmas are submerged within the anther tube or are found at the mouth of the tube so that they will automatically be self-pollinated as pollen drifts through the anther tube. Occasional plants whose stigmas are consistently exserted under conditions at Davis, California have been observed by the writer to be as unfruitful as male-sterile mutants. In all wild forms seen by the writer in Perú and Ecuador the stigma appears either outside the anther tube or, less frequently, at the mouth of the tube. When first cultivated in areas of low vector activity, therefore, the tomato may have been subjected to intense but probably unintentional selection for stigmas contained within the anther tube. This conclusion must remain tentative until we know more concerning the relative importance of heredity and environment upon stigma position. The present study provides one example of the effect of environment. Genetic control is demon-

strated in the work of Currence (1944) and also by the fact that interspecific differences in the degree of exsertion are maintained in culture under the same environment.

Another problem raised by the change in pollination relations is the effect on breeding systems and its genetic consequences. The ability to cross-pollinate probably conveys great advantages to the tomato growing in its native habitat. A larger reserve of variability is thereby maintained, from which the species can draw when confronted with changes in the environment. Facultative cross-pollination also permits the spread of favorable genes and the opportunity to combine them with other genes to synthesize better adapted genotypes. These advantages become less important when the plant is cultivated because man will select the types that are best adapted to the conditions of his garden and can artifically cross-pollinate them when he sees the need for combining characters of different lines. On the other hand, the inbreeding consequent to the self-pollination imposed by cultivation in new areas may have served to expose and fix recessive traits that may not have been known or fixed before the transfer. The homozygosity that was an inevitable consequence of this change was probably appreciated by the early plant breeders as it is by the modern ones. The origin of diverse forms and the accumulation of the many recessive genes, by which our modern tomatoes differ from related wild types, is therefore easily understood.

The realization of a possible change in the breeding systems may likewise aid our understanding of the frequently demonstrated heterosis that is manifest in the hybrids between different tomato varieties. Accepting the theory that the degeneration wrought by inbreeding results from the fixation of deleterious recessive genes and that the heterosis in hybrids between inbred lines stems from the "covering" of deleterious recessives of one parent by favorable dominants of the other, Dobzhansky (1946) argues that little heterosis would be expected in self-fertilizing species, in which "deleterious recessive mutants and deleterious recombination products of the gene complexes are eliminated by natural selection almost as rapidly as deleterious dominants." Furthermore, regardless of the theory of heterosis assumed, if hybrids between individuals of a naturally self-fertilized species were more vigorous than non-hybrid individuals, mechanisms that ensure cross-fertilization would tend to replace, or to supplement, self-fertilization in nature unless the selective advantages of hybrid vigor were outweighed by other features of self-fertilization. A selective advantage might be imagined, for example, in the guarantee of seed production if populations are very small or if pollen vectors are lacking, or in the opportunity to fix genotypes that are highly adapted to a very uniform environment as in the case of certain annual weeds. *L. esculentum* does not present any such dilemma because it has been demonstrated here to be highly cross-pollinated in its native habitat. The self-pollination imposed by cultivation in non-native regions resulted in rapid inbreeding, presumably with some loss in vigor, and our older tomato varieties consequently are pure lines or collections of very closely related pure lines. Admittedly newer varieties derived from recent hybridization may still segregate, but they will inevitably reach the pure line condition. Heterosis in hybrids between tomato varieties therefore is not surprising because these are hybrids between what are, in effect, inbred lines of a naturally cross-pollinated species.

These considerations are intimately related to the domestication of the tomato and its subsequent transfer to the Old World. In this respect it would be desirable to know the pollination mechanisms in Mexico, which country Jenkins (1948) considers on the basis of many kinds of evidence to be the most likely site of its domestication as a cultivated plant. A hint that tomatoes may also be

considerably cross-pollinated in Mexico is given by the fact that certain Mexican varieties grown in California have stigmas so greatly exserted that they set few fruit in the field. If this is generally true of Mexican varieties the changes in the breeding system just discussed must have occurred in Europe after the tomato had been introduced from the New World.

Summary

Rates and other features of natural cross-pollination of *Lycopersicon esculentum* in stations within the range of wild forms in Perú were studied by counting the number of seeds and fruits produced by male-sterile mutants and were compared with similar data previously obtained in California, where no species of Lycopersicon are native.

Useful data, which were provided by two plots, both located within the range of distribution of *L. esculentum, L. pimpinellifolium,* and *L. peruvianum,* showed much higher rates of natural cross-pollination than were obtained in any tests outside the range of distribution. Mean values of 14.2 and 14.8 seeds per flower, construed to be the most useful single measure of cross-pollination, for the Peruvian plots contrast with numbers varying from 0.3 to 7.0 for 16 plots in California. Expressed as a proportion of the fertile-plant yield, the values for Perú are respectively 41.4 and 51.4 per cent and for California vary from one to 33 per cent. The interbranch and interplant variability is generally lower in the Peruvian than in the Californian data.

The appearance of natural hybrids between varieties of *L. esculentum* and between this species and *L. pimpinellifolium* and also the much greater activity of insect pollen vectors observed in Perú lends support to the direct measures of natural cross-pollination.

The insects visiting flowers of tomatoes were collected and the identifications by P. H. Timberlake are presented herewith. *L. esculentum* in the region studied is actively pollinated by two species of native solitary bees. Bees of nine other species representing diverse families were obtained in cursory collections from other species of *Lycopersicon.*

The results are discussed in relation to (1) commercial production of F_1 hybrid seed, (2) the change in breeding system during the historical transfer of *L. esculentum* to non-native areas from a facultatively cross-pollinated species to an almost exclusively self-pollinated one, and (3) the bearing of the change in pollination relations on flower form, heterosis, and appearance of mutants.

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MATING BEHAVIOR IN *DROSOPHILA AFFINIS* AND *DROSOPHILA ALGONQUIN*

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INTRODUCTION

As has been reported by numerous authors (e.g. Sturtevant, 1921; Stalker, 1942; Mayr, 1946; Wallace and Dobzhansky, 1946; and Spieth, 1949), when males of one species of *Drosophila* are mixed with females of another, mating may not take place or may take place only with difficulty. Even where no striking differences are discernible between the normal mating behaviors of the two species, there may be noticeable difficulty in interspecific mating (e.g. the combination of *D. pseudoobscura* and *D. persimilis;* Mayr, 1946). In some cases in which mating is reduced, either the males or the females may behave in such a manner as apparently to interfere with successful crossing. For example, Sturtevant (1921) attributes the reduction of interspecific mating between *D. melanogaster* and *D. simulans* chiefly to the females, since the males seem to court females of either species indiscriminately. On the other hand, certain observations of interspecific mixtures have shown some disinclination of *Drosophila* males to mate with females of alien species. Stalker (1942) reports some hesitancy on the part of *virilis* and *americana* males when confronted with alien females, and Spieth (1949) reports sudden cessations of courtship by males in certain interspecific combinations of *D. willistoni* group species. It was the object of the present study to extend observations of mating behavior to the North American species *D. affinis* and *D. algonquin* and, in particular, to observe this behavior in mixtures of males of one species with females of the other.

Drosophila affinis Sturtevant, 1916, and *Drosophila algonquin* Sturtevant and Dobzhansky, 1936, are similar species belonging to the *obscura* group (*affinis* subgroup) of the subgenus *Sophophora* (Sturtevant, 1942). Both are widely distributed in the United States east of the Rocky Mountains, and the distributions of the two species overlap in the western Great Plains and in the northeastern states. These species are morphologically quite similar, though males of the two species may be easily distinguished by the sex combs, which are small in *affinis,* large in *algonquin* (Sturtevant and Dobzhansky, 1936). Presumably these species are somewhat similar ecologically, since they occur together in some localities and may be collected in the same fermented banana traps. Both may be cultured quite readily in the laboratory. Attempts to hybridize these species have not been successful, and interspecific insemination has not been observed to take place between them. However, it has been possible to hybridize females of both *affinis* and *algonquin* with males of a third *affinis* subgroup species, *D. athabasca* Sturtevant and Dobzhansky, 1936, which is found in northern North America and which overlaps the ranges of *affinis* and *algonquin* in the northeastern United States (Miller, 1941). (Unfortunately, a vigorous strain of *athabasca* was not available at the time of this study, so it was not possible to make detailed observations of mating behavior in this species. It is planned to do so as soon as such a strain can be established.)

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[2] Contribution Number 245 of the Department of Zoology and Anatomy of the University of Nebraska.

Materials and Methods

Most of the mating observations were made with one strain of *D. affinis* and with another of *D. algonquin*. The strains chosen for this study both originated in southeastern Nebraska. The *affinis* strain, Huskerville-1, was derived from a female collected at Huskerville (about seven miles northwest of Lincoln) in 1948 by Mr. A. A. Russell, while the *algonquin* strain, Lincoln-2, was started from a female collected in Lincoln in 1949 by Mr. A. Yanders. These strains were both quite vigorous under the laboratory conditions, and flies of the two species taken from these strains were of about the same size (with only a slight tendency of *algonquin* to be larger than *affinis*). In addition, a few observations were made with *affinis* strains Henry-1 (originated by Mr. Russell from a female obtained at Henry, Nebraska, near the Wyoming border) and Woods Hole-4 (obtained from the collection of Professor A. H. Sturtevant of the California Institute of Technology) and with hybrids between these two strains. Since mating behavior in these other strains of *affinis* was not significantly different from that of the Huskerville-1 strain, it was decided to restrict most of the observations of *affinis* to the latter strain.

The *Drosophila* stocks were cultured in half pint milk bottles on cornmeal-molasses-agar-moldex medium, and the flies were raised in an incubator in which the temperature ranged from 22° to 24.5° C. Adult flies to be used in the mating observations were isolated each morning, with the collection times about twenty-four hours apart (sufficiently frequent for the collection of virgins in these species). The males and females to be mated were aged separately in shell vials for ten days. Although mating will take place in both *affinis* and *algonquin* within a few days after eclosion, it was decided arbitrarily to age the flies ten days in the hope of getting fairly high intensities of mating activity. Usually twelve flies were put in an ageing vial, but sometimes smaller numbers were used. Food during the ageing period consisted of a rectangular strip of heavily yeasted culture medium on a cardboard parallelogram inserted into the vial (a modification of the culture method of Spencer, 1943). The food strips were renewed after three, six, and nine days. During the ageing period the vials were kept in the incubator in which the flies were raised.

In order to observe mating behavior, males and females were mixed without etherizing in shell vials of the kind used for ageing. These vials were about 9 centimeters long and had a diameter of about 2 centimeters, and, with the cotton plug inserted, the flies were confined in a volume of about 22 cubic centimeters. In the early observations the females were mated in their own ageing vials, the males being introduced into the females' vials after the food strip had been removed. Later, because an appreciable amount of moisture condensed in the ageing vials (accompanying increased atmospheric humidity), the females were transferred to clean vials before mating. No apparent alteration of mating behavior accompanied this change of procedure. As little shaking as possible was used to transfer the flies. In order to promote movement of the flies from one vial to another, the vial into which the flies were being transferred was regularly held towards a source of light and elevated a little. Flies that were very reluctant to leave their vials were left behind and thereby excluded from the matings.

Mating observations were generally made in the morning between 6 and 10 A.M., with a few exceptions. These observations were made in a room where the temperature was observed to vary from 21° to 30° C., usually being higher than in the incubator where the flies were raised and aged. The vials in which the flies had been mixed for mating were placed on the stage of a dissecting microscope (occasionally more than one vial

at a time). A desk lamp was used as a light source, with a flask of water in front of it to prevent overheating of the vials. Each mating vial was observed for a half an hour. Most of the observation was made with the naked eye, but a few details were observed with the low power of the microscope (10.5 diameters).

When the observation period was over, the flies in the mating vial were etherized and counted. Due to the fact that some of the flies died or were otherwise lost during the ageing period and also because some of them were occasionally left behind in their vials, the numbers of males and females participating in the matings

TABLE 1. *Courtships in* D. affinis (*Huskerville-1*)

Date	Time	Temp.	Combination	Observed courtships			Excess	χ^2
				♂–♂	♂–♀	Total		
5 July	0728	30° C.	12♀♀ × 10♂♂	21_5	30_7	51	♂–♀	0.079
6 July	0641	29	10♀♀ × 8♂♂	8	4_2	12	♂–♂	3.086
7 July	0641	29	11♀♀ × 10♂♂	49_2	31_{14}	80	♂–♂	8.258 S
9 July	0649	27	12♀♀ × 9♂♂	8	19_5	27	♂–♀	1.381
10 July	0700	27	8♀♀ × 10♂♂	22_3	13_5	35	♂–♂	1.037
13 July	0841	26	9♀♀ × 7♂♂	1	7	8		
13 July	1324	27	9♀♀ × 4♂♂	0	4	4		
14 July	0642	24	10♀♀ × 7♂♂	8	27_3	35	♂–♀	3.059
14 July	0932	25	8♀♀ × 8♂♂	2	7_2	9		
16 July	0728	25	10♀♀ × 10♂♂	11	16_7	27	♂–♀	0.601
30 July	0716	21	9♀♀ × 10♂♂	17_1	12_1	29	♂–♂	0.534
10 August	0716	28	7♀♀ × 10♂♂	4_1	3_1	7		
13 August	0744	28	9♀♀ × 9♂♂	8_1	5_4	13	♂–♂	1.238
14 August	0702	26	9♀♀ × 9♂♂	6	6_4	12		0.000
15 August	0820	27	6♀♀ × 6♂♂	2	9_5	11	♂–♀	3.300
15 August	0909	28	7♀♀ × 9♂♂	13	11_1	24		0.000
			146♀♀ 136♂♂					

TABLE 2. *Courtships in* D. algonquin (*Lincoln-2*)

Date	Time	Temp.	Combination	Observed courtships			Excess	χ^2
				♂–♂	♂–♀	Total		
7 July	0658	29° C.	10♀♀ × 9♂♂	33	42_{11}	75		0.000
7 July	0741	30	10♀♀ × 12♂♂	37_1	24_7	61	♂–♂	1.643
9 July	0807	27	7♀♀ × 7♂♂	30	30_5	60	♂–♂	0.606
10 July	0911	28	9♀♀ × 6♂♂	8	15_5	23		0.000
13 July	0858	26	9♀♀ × 6♂♂	19	30_6	49	♂–♂	0.088
13 July	1405	28	8♀♀ × 5♂♂	7	19_7	26	♂–♀	0.679
14 July	0726	24	12♀♀ × 6♂♂	3	16_{10}	19	♂–♀	3.562
16 July	0808	26	4♀♀ × 2♂♂	7	11_6	18		
30 July	0814	22	6♀♀ × 12♂♂	35_1	18_9	53	♂–♂	0.082
10 August	0808	28	8♀♀ × 6♂♂	4	15_3	19	♂–♀	2.036
10 August	0809	28	6♀♀ × 7♂♂	11	9_4	20	♂–♀	0.200
13 August	0834	28	6♀♀ × 5♂♂	7_1	7_2	14	♂–♂	0.292
14 August	0703	26	9♀♀ × 7♂♂	50	24_{10}	74	♂–♂	22.424 S
14 August	0755	25	9♀♀ × 10♂♂	26_2	38_{13}	64	♂–♀	2.250
15 August	0822	27	6♀♀ × 6♂♂	3	2_1	5		
15 August	0910	28	7♀♀ × 6♂♂	5	15_1	20	♂–♀	1.875
			126♀♀ 112♂♂					

TABLE 3. *Courtships in combinations of* D. algonquin (*L-2*) *females and* D. affinis (*Hk-1*) *males*

Date	Time	Temp.	Combination	Observed courtships			Excess	x^2
				♂-♂	♂-♀	Total		
6 July	0755	29° C.	7♀♀ × 6♂♂	6_2	9	15		0.000
7 July	1122	30	10♀♀ × 9♂♂	12_1	2	14	♂-♂	10.500 S
8 July	0713	29	11♀♀ × 9♂♂	5_1	4	9		
10 July	0740	29	6♀♀ × 8♂♂	23	12	35	♂-♂	1.842
15 July	0943	26	3♀♀ × 6♂♂	2	1	3		
20 July	0854	27	3♀♀ × 6♂♂	3	0	3		
30 July	0716	21	9♀♀ × 12♂♂	5	8	13	♂-♀	1.238
4 August	0824	25	7♀♀ × 10♂♂	10	0	10		
9 August	0733	27	8♀♀ × 12♂♂	17	4	21	♂-♂	4.861 S
9 August	0824	27	7♀♀ × 9♂♂	15_1	5	20	♂-♂	3.233
10 August	0807	28	8♀♀ × 10♂♂	2_2	1	3		
11 August	0710	27	8♀♀ × 8♂♂	21_4	5	26	♂-♂	12.536 S
11 August	0803	27	8♀♀ × 11♂♂	10	6	16		0.000
12 August	0837	29	7♀♀ × 11♂♂	31_4	6	37	♂-♂	9.082 S
12 August	0838	29	8♀♀ × 8♂♂	23_3	18	41	♂-♂	1.569
13 August	0745	28	8♀♀ × 11♂♂	25_1	8	33	♂-♂	5.989 S
			116♀♀ 146♂♂					

TABLE 4. *Courtships in combinations of* D. affinis (*Hk-1*) *females and* D. algonquin (*L-2*) *males*

Date	Time	Temp.	Combination	Observed courtships			Excess	x^2
				♂-♂	♂-♀	Total		
6 July	0832	29° C.	7♀♀ × 3♂♂	14_2	4	18		
7 July	0856	29	11♀♀ × 8♂♂	87_1	10	97	♂-♂	103.879 S
8 July	0750	29	10♀♀ × 6♂♂	47_1	4	51	♂-♂	79.412 S
10 July	0828	28	9♀♀ × 7♂♂	35	1	36	♂-♂	51.546 S
20 July	0736	26	8♀♀ × 6♂♂	20	5	25	♂-♂	16.667 S
25 July	0716	28	12♀♀ × 8♂♂	13	2	15	♂-♂	13.611 S
27 July	0700	28	9♀♀ × 6♂♂	5	3	8		
30 July	0813	21	10♀♀ × 7♂♂	2	3	5		
4 August	0823	25	7♀♀ × 6♂♂	30	5	35	♂-♂	13.931 S
8 August	0855	28	12♀♀ × 8♂♂	14	5	19	♂-♂	11.083 S
9 August	0822	27	10♀♀ × 6♂♂	10	3	13		
9 August	0822	27	10♀♀ × 7♂♂	15_1	4	19	♂-♂	14.476 S
10 August	0715	28	11♀♀ × 6♂♂	12_1	3	15		
11 August	0804	28	11♀♀ × 9♂♂	12	6	18	♂-♂	3.600
11 August	0805	28	12♀♀ × 8♂♂	38_1	5	43	♂-♂	48.176 S
12 August	0740	28	9♀♀ × 9♂♂	23	0	23	♂-♂	25.091 S
12 August	0836	29	10♀♀ × 5♂♂	5_1	3	8		
13 August	0832	28	8♀♀ × 4♂♂	2	0	2		
			176♀♀ 119♂♂					

were quite variable. A record of most of the observations on which this report is based is given in tables 1 through 4, with the total numbers of flies used in these observation (dates all summer, 1949).

TABLES 1–4. *Numerical record of the mating observations.* The beginning times of the half hour observation periods are given. The subscripts to the numbers of male-male courtships are the numbers of times males were observed

to attempt copulation with other males. The subscripts to the numbers of male-female courtships include both attempted and successful copulations of males with females. The derivation of the Chi-square values is given in the text. In each case where a Chi-square calculation was made, it is indicated whether male-male or male-female courtships were in excess. Significant and highly significant deviations are indicated by an "S".

Results of the Observations

During this study a difference in sensitivity to handling was apparent between *affinis* and *algonquin*. Under the prevailing conditions, *affinis* was definitely more responsive to changes in its environment than was *algonquin*. This was apparent during the ageing period at the times of changing of food and also when the flies were mixed for mating.

When food was changed in the ageing vials, it was the practice to hold a vial with its closed end towards a light source and to shake it a little so the flies would go to the closed end and the vial could be unplugged and the food strip replaced. With *affinis*, response to this treatment usually took place quickly, the flies leaving the food and going to the end of the vial, where they moved about in an excited manner. *Algonquin*, on the other hand, was regularly more sluggish. Flies of this species often responded very slowly to the orientation and shaking of the vial, and frequently it was necessary to make individuals move away from the food by prodding them with a blunt needle. (This kind of behavioral difference between *affinis* and *algonquin* had been reported to the author by Mr. A. A. Russell before these experiments began.)

The greater sensitivity of *affinis* was still more apparent when the males and females were mixed for mating. In *affinis*, transferring and mixing were almost invariably followed by an appreciable amount of movement which may be described as uncoordinated. The flies would jump about aimlessly, sometimes gliding along the glass buzzing their wings, and occasionally coming to lie on their backs, either struggling or lying still, sometimes remaining quiet several minutes. (An apparently similar reaction to mechanical disturbance has been reported by Stalker and Spencer, 1939, in *D. macrospina*, in which these authors describe "catyleptic fits.") This uncoordinated movement often lasted a number of minutes, and during this period of activity little if any courtship would take place. In a few cases, such uncoordinated activity lasted intermittently throughout the entire observation period. The intensity of this activity was variable, apparently depending to a certain extent on unknown internal factors. Although rough treatment seemed to increase this activity, it was not possible to eliminate it entirely by exercising care in handling the flies. In *Algonquin*, on the other hand, only a little uncoordinated activity was observed, and mating behavior usually began soon after males and females of this species were mixed.

Mating Behavior in Intraspecific Combinations

Courtship in *D. affinis* was described by Sturtevant (1921) before the recognition of *algonquin* and the other *affinis* subgroup species. The present observations of both *affinis* and *algonquin* are in general agreement with those of Sturtevant. The mating behaviors of these species were found to be very similar. Consequently the following description may be taken to apply to both these species, except where otherwise indicated.

In *affinis* and *algonquin*, courtship is usually very brief, being followed quickly by copulation, though it may sometimes be fairly protracted. The following stages of courtship and copulation may be recognized: orientation of the male to the individual to be courted; vibration of the wings of the male, usually accompanied by circling of the male about the courted individual; attachment of the genitalia (initiation of copulation), with partial

mounting; and completion of mounting. The early part of courtship in these species does not necessarily involve physical contact between individuals (such as the "tapping" described by Rendel, 1945, in *D. subobscura;* and by Spieth, 1947, in species of the *D. willistoni* group). However, in *affinis* and *algonquin,* extensions of the fore- and middle legs, with occasional touching, may sometimes be seen in flies standing close to each other. Such movements have been described in various species of *Drosophila* (e.g. Stalker, 1942; Spieth, 1947) and appear not to be necessarily a part of courtship.

Orientation of sexually active males of *affinis* and *algonquin* is usually followed quickly by wing vibration. Males of these species frequently orient and vibrate to other males as well as to females, and they have also been observed to go through these movements before dead males and females. Orientation usually seems to result from chance contacts as the flies move about. Orientation may be towards any part of the courted individual's body, and sometimes the male moves sidewards quickly from one end of this individual to the other (circling). Wing vibration by *affinis* and *algonquin* males is usually done with both wings spread about 90° apart or more. In this respect these species are different from certain similar species in which the males vibrate only one wing at a time (e.g. *pseudoobscura,* Mayr, 1946). Occasionally at the height of sexual activity males of *affinis* and *algonquin* were seen to rush about vibrating their wings without being oriented to any other individual.

In response to courtship, females react in various ways, apparently depending on their disposition towards copulation. A female may stand still and permit the courting male to attach his genitalia and mount her. On the other hand, the female may show obvious avoiding movements, turning in such a way as to keep the male from her posterior or moving quickly away, running or flying. Another response of courted females consists of an extension of the abdomen and extrusion of the genitalia in the direction of the courting male. Occasionally an egg protrudes from the ovipositor. Such a movement has been described in other *Drosophila* species (e.g. Mayr, 1946, in *pseudoobscura;* Spieth, 1947, in the *willistoni* group species). However, although Mayr describes the movement as an "invitation display," usually followed by copulation, Spieth refers to it as a means employed by nonreceptive females to repel courting males. In *affinis* and *algonquin,* it is the impression of the author that extrusion of the genitalia by females serves to prevent mating. This gesture is a regular response of females that have recently copulated, and females of these species were not observed to accept a male soon after copulation. Males usually desist from courting females that extrude their genitalia towards them. Males that are courted by other males either avoid the courting male by departing quickly or they turn on the male and court him in return. Males sometimes court each other with considerable vigor. Several times pairs of males were observed vibrating to each other, sometimes chasing each other in a circle, each attempting to approach the other's posterior.

Attachment of the genitalia and partial mounting (foretarsi on the abdomen of the courted individual) take place almost simultaneously. Although certain females that proved to be receptive were seen to spread their wings slightly as a courting male approached, males were also seen to copulate with females that did not spread their wings. Occasionally a male would have difficulty attaching his genitalia and would spend several seconds partially mounted on the female, frantically buzzing his wings during this time. Some such mating attempts apparently failed, and the male departed without having completely mounted. It was not determined whether such brief encounters are ever accompanied by insemination. Usually attachment of the genitalia is accomplished quickly, and the male climbs on

the female, shoving her wings apart. The mounted male regularly assumes a position well forward on the female's abdomen, with his foretarsi near the axils of the female's wings. Males sometimes attempt copulation with other males. In a very few cases the courting male was observed to mount the other male completely, with some kind of attachment at the genital region. The nature of such attachments was not determined, and all of them were very brief.

During copulation the male and female usually stand quite still, the abdomen of the female drawn up under the male's abdomen, which contracts rhythmically. Termination of the copulation appears to depend on the male. Towards the end of the copulation the male spreads and vibrates his wings slightly several times, detaches his genitalia while still occupying his position on the female's back, and then hops off. Sometimes following detachment of the genitalia a male remains on the back of the female a number of seconds (sometimes as long as two minutes in *affinis*). In several cases there was observed to be some difficulty in separating the genitalia. In these cases the male turned around about 180°, and if the genitalia did not then come apart, both members of the pair struggled until separation was effected.

Affinis and *algonquin* were found to differ in duration of copulation, with copulation always being briefer in *affinis* than in *algonquin*. In *affinis*, 36 copulations were timed. The lengths of these ranged from 39 seconds to 2 minutes 29 seconds, with an average of 1 minute 14 seconds. In *algonquin*, 42 copulations were observed to range from 3 minutes 29 seconds to 11 minutes 35 seconds, with an average of 5 minutes 41 seconds. (These figures do not include lengths of copulations in which there was obvious difficulty of separation, nor do they include the times spent by some males on the females' backs following separation of the genitalia.) Figure 1 shows the distribution of copulation times with respect to temperature. A marked negative correlation with temperature appears to exist in *algonquin* above 24°, and a slight negative correlation is suggested for *affinis* for the entire temperature range. Since the flies were mixed in groups, not all of the copulations were known to be first copulations. Those known to be first copulations for both participating individuals are indicated as such in figure 1.

Following copulation, the male and female usually stand still a little while, each rubbing its genital region with the hind legs. The male may begin courting again and may copulate if permitted to do so (one *algonquin* male was observed to mate twice during 15 minutes). The recently mated female, as already pointed out, refuses the advances of courting males, and there was no clear case of a female's mating twice during the observation period. However, Sturtevant (1921) reported that a pair of *D. affinis* was seen to mate twice in 10 minutes.

The intensity of mating activity varied from one observation vial to another. There was no obvious correlation between this activity and any observed environmental component, such as temperature or time of day. However, certain factors difficult to measure (e.g. mechanical disturbance) may have been important. In some of the mating vials there were seen to be marked individual differences between males with regard to frequency of courtships, and it seems likely that part of the basis for variation in mating activity in the different vials was due to such individual differences. As already remarked, the high excitability of *affinis* usually caused some delay in mating activity in this species.

The impression was obtained that males of *affinis* and *algonquin* courted other males with such high frequencies that, regarding the initiation of courtship, little if any sex discrimination on the part of these males might be inferred. An attempt was made to count the male-male and male-female courtships (orientation and wing vibration together being taken

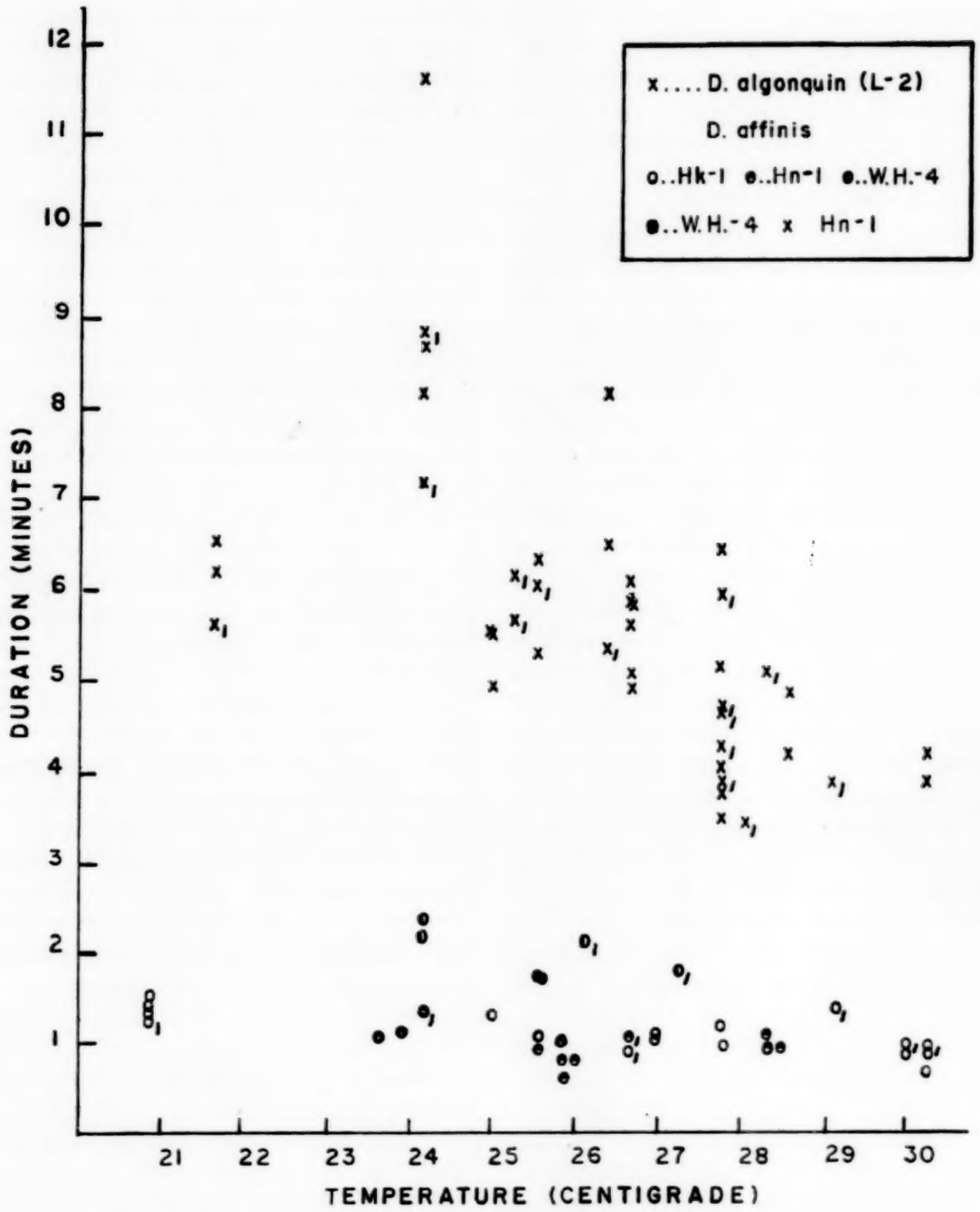

FIG. 1. Lengths of copulation in *D. affinis* and *D. algonquin* plotted against temperature. A subscript number "1" indicates that the copulation was known to be the first copulation for the participating individuals.

as the criterion of courtship). Although certain errors in making these counts must have occurred, especially when mating activity was high, it was felt that the numbers obtained this way should give a satisfactory representation of the relative frequencies of the two kinds of courtships. The numbers of courtships counted in the intraspecific combinations of *affinis* and *algonquin* are presented in tables 1 and 2 respectively.

Since the numbers of males and females varied in the different observation vials, the frequencies of chance contacts of males with individuals of the two sexes should have varied from one vial to another.

Consequently, the numbers of courtships of the two kinds, which seemed to depend on chance contacts, cannot be compared directly from one observation to another. As a basis for comparison, the observed frequencies of male-male and male-female courtships of each observation have been tested by the Chi-square method for agreement with the expected frequencies based on chance contacts (except where the numbers of courtships were so low that either expected class was less than 5). The expected frequencies of male-male and male-female courtships were made proportional to the numbers of potential pairs of the two kinds—the number of male-male pairs being the square of the number of males minus the number of males, while the number of male-female pairs was the product of the number of males by the number of females. As may be seen in tables 1 and 2, in the majority of cases deviation from the expected frequencies was not significant. However, heterogeneity Chi-square values determined for the data of tables 1 and 2 respectively were found to be so very large that the data of neither table can be considered homogeneous enough to warrant evaluation of the pooled data. Consequently, the totals of the two kinds of courtship (with their Chi-square) are not given in either table, and the Chi-squares presented are intended, as indicated above, only as a basis for comparison.

Although it may be concluded that the frequency of male-male courtship is conspicuously high in *affinis* and *algonquin*, it cannot be considered established that sex discrimination on the part of males of these species is completely lacking, even for the initiation of courtship. One factor which must have generally augmented the numbers of male-male courtships was the tendency of males to respond to courtship by courting in return. In some cases it was quite apparent that a courtship of one male by another was followed by a series of male-male courtships, with first one male courting and then the other, with brief intervals between courtships. (However, because it was not always possible to determine the dependence of a male-male courtship on an earlier one, these cases were not separated in the data.) Especially where mating activity was intense, such series of male-male courtships were observed. It seems likely that they were largely responsible for those cases of frequencies of courtships between males so great as to give large deviations from expectations (*affinis*, 7th of July; *algonquin*, 14th of August). In addition, a certain degree of sex discrimination on the part of *affinis* and *algonquin* males is suggested by the numbers of attempted and successful copulations (both male-male and male-female), although these numbers are very small (subscripts in tables 1 and 2). It may be seen that in no case was the number of attempted and successful pairings of males with females exceeded by the number of attempted male-male copulations.

Courtships of males by males has also been reported in other species of *Drosophila* and in other insects, although in some of these it appears to be less common and less vigorous than in *affinis* and *algonquin*. Sturtevant (1915) reports that although males of *D. melanogaster* do not ordinarily court each other, males of this species were observed to court males that had recently paired with females and would court dead males, especially if these had had the body juices of females put on them. *D. melanogaster* males that had been kept in solitary confinement were also found to court each other. Sturtevant also mentions cases of intermale courtship in other insects, including reference to a species of *Psychoda* in which male-male courtship is common, with frequent attempts at copulation between males. Streisinger (1948) reports that *D. pseudoobscura* males confined with etherized females courted each other a great deal and tended to avoid the quiet females. Spieth (1949) reports intermale courtship in certain *willistoni* group species following the introduction of fe-

males (conspecific or alien) into groups of males.

In *affinis* and *algonquin*, the manifestation of courtship behavior in males does not depend on the presence of females, nor even on recent association with females, since the males of these species were frequently observed courting each other in the isolation vials before being mixed with the females. On the other hand, isolation from females for a period of time is not a requirement for courtship of males by males. Males of these species that were aged with females for ten days under the usual conditions were observed to court each other as well as the females. Male-male courtship was also observed in both *affinis* and *algonquin* with only four days of ageing (very little mating activity could be observed with still younger flies). A few *D. melanogaster* males and females (Canton-S strain) aged in isolation for ten days and then mixed showed quite different mating behavior from that observed in *affinis* and *algonquin*. Here the males courted the females most vigorously, and no case of male-male courtship was observed.

Mating Behavior in Interspecific Combinations

Observations were made of both reciprocal combinations of *affinis* and *algonquin* in which males of one species were mixed with females of the other. As in the intraspecific mixtures, an attempt was made to count male-male and male-female courtships. The numerical data from these observations are given in tables 3 and 4, with Chi-square values derived as before (here, also, the heterogeneity Chi-squares were very large, so the courtship totals and their Chi-squares are not presented).

In the interspecific combinations, although the males courted other males with considerable vigor, they courted the alien females only briefly if at all, and there were no cases of attempted copulations of males of one species with females of the other. Frequently the males would seemingly orient to the females without vibrating their wings (such movements were not recorded as courtships). Often the males would approach the females vibrating their wings and then suddenly turn away. Only very rarely did the males come in physical contact with the females, and this occurred apparently only by accident. On the other hand, the males courted each other frequently and sometimes atempted copulation with each other (as indicated by the subscripts in tables 3 and 4). This apparent preference of males for other males of their own species rather than alien females was especially striking in the combination of *affinis* females with *algonquin* males (table 4). This must partly have been due to the fact that the *algonquin* males were generally able to show somewhat greater mating activity than the *affinis* males under the conditions of these observations. However, the greater excitability of *affinis* did not in itself seem to serve as a barrier to interspecific mating. For example, even when the *affinis* females stood quite still, the *algonquin* males refused to attempt copulation with them. The existence of relatively high frequencies of male-male courtships in these interspecific combinations is shown by the generally large Chi-square values of tables 3 and 4, representing deviations which in all but one case (*algonquin* females by *affinis* males, 30 July) involve excesses of male-male courtships.

Discussion

Following the example of other observers of insect behavior (e.g. Spieth, 1947 and 1949), one may interpret mating behavior in *affinis* and *algonquin* as a series of actions brought about by certain stimuli. The initiation and maintenance of mating behavior in the males of these species may be considered to depend on stimuli some of which are sufficient to cause courtship to begin while others are necessary for the continuation of courtship. The beginning of courtship (orientation and wing vibration) has been seen

to come about in the presence of several different kinds of individuals—conspecific males and females (including dead individuals of these kinds) and females of the alien species. (In addition, *affinis* and *algonquin* males have been seen to court females of *D. melanogaster*.) However, courtship by males of these species is noticeably less vigorous with alien females than with conspecific individuals, either males or females. The courtships of the alien females are always very brief, and copulation and mounting are not attempted. From this it may be concluded that stimuli necessary for the continuation of courtship are sufficiently different in the two species so that males that begin courting alien females are not stimulated in such a way as to cause them to carry their mating behavior to completion.

Since the males of *affinis* and *algonquin* do not come into direct contact with the alien females, but turn away from them without attempting to copulate, the stimuli that these males receive, and which cause them to stop courting, must operate at a distance. The nature of such stimuli remains obscure. Spieth (1949) suggests that chemoreception is probably responsible for the failure of mating between *D. willistoni* females and *D. capricorni* males, which often break off courtship without having come into direct contact with the females. It is quite possible that chemical stimuli operating at a distance are responsible for species discrimination in the combinations of *affinis* and *algonquin*.

Summary

The mating behaviors of *D. affinis* and *D. algonquin* were found to be very similar. Courtship in both species consists of orientation, wing vibration, and circling of the courting male about the courted individual. Males seem to court males about as frequently and as vigorously as they court females. A difference of duration of copulation was observed between the two species, with copulation in *affinis* regularly being of shorter duration (average 1 minute 14 seconds) than in *algonquin* (average 5 minutes 41 seconds). A negative correlation between length of copulation and temperature was suggested for both species in most of the temperature range. In combinations of males of one species with females of the other, although the males court each other with considerable vigor, they court the alien females only briefly if at all and do not attempt copulation with them. The discrimination of males of one species against females of the other appears to be due to stimuli received at a distance.

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NATURAL SELECTION FOR REPRODUCTIVE ISOLATION BETWEEN *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS*[1]

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INTRODUCTION

One of the most important problems in the study of speciation has been that of the origin of reproductive isolating mechanisms, for it is by the building up of intrinsic barriers which prevent gene exchange between populations that we pass from the racial or subspecific to the specific level. Two theories have been proposed to explain how reproductive isolation might arise. The first, favored by Muller (1939, 1942), holds that reproductive isolation is a by-product of genetic divergence. As two subspecies differentiate in geographical isolation, genes which hamper free interbreeding with the other subspecies are incorporated into the genotype in the course of building up adaptive gene complexes. The reproductive isolation may be a pleiotropic effect, or perhaps be a result of change in gene function.

The other theory, advanced by Dobzhansky (1940), holds that provided the two subspecies have diverged from one another far enough so that hybrids between them are less well adapted for any available habitat than either parental type, natural selection will act to build up further reproductive isolation, thus preventing the formation of inadaptive hybrids with consequent wastage of reproductive potential and food resources. These two theories are by no means mutually exclusive, and indeed both mechanisms in all probability play an important part, perhaps being of greatest importance at different stages in the process. Actually, it seems very difficult to conceive of a situation in which the development of isolating mechanisms between two populations could proceed entirely by means of selection, since this would mean that at the outset, when selection is to begin its action, the hybrid and the two parental types would be equally viable and fertile and the former would be at no selective disadvantage. Under these circumstances, however, natural selection against the hybrid would be impossible. On the other hand, it is exceedingly probable that two populations which, during genetic divergence, had developed a considerable amount of reproductive isolation, would upon physical contact complete the process by means of natural selection.

Unfortunately, to date, the evidence, experimental or otherwise, showing either mechanism at work is virtually absent. The experiments herein described were made in order to determine whether, in artificial populations consisting of the two closely related species, *Drosophila pseudoobscura* and *D. persimilis* (the latter formerly known as *D. pseudoobscura*, race B), an increase in the reproductive isolating mechanisms could be detected if in each generation the hybrids between the two species were systematically eliminated. Under these conditions, if any hereditary variability for reproductive isolation was present, natural selection should act to prevent these hybrids from being formed.

MATERIALS AND METHODS

In order that not only representatives of the pure species, *D. pseudoobscura* and *D. persimilis*, but also both male and fe-

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male hybrids could be easily recognized, mutant stocks of both species were used. For the *D. pseudoobscura* stock, males of the second chromosome mutant *glass* were outcrossed to a recently captured strain from Jacksonville, California, and the *glass* mutant reextracted. For the *D. persimilis* stock, males of the third chromosome recessive mutant *orange* were outcrossed to a recently captured strain from Porcupine Flat, California, and the *orange* mutant reextracted. This outcrossing and reextraction was done for two reasons. First, the viability of the strains was improved by heterosis, as well as by introduction of genes which had been under strong natural selection in the wild, as opposed to the relatively weak natural selection experienced by the mutant strains in the laboratory. Secondly, since mass cultures were employed, the store of genetic variability was increased, an important factor in the present experiment. By using these mutant stocks, all *D. pseudoobscura* individuals could be recognized by having *glass* eyes, all *D. persimilis* by being *orange*-eyed, while the only wild-type individuals were the hybrids, in which both mutant genes were covered by the wild-type alleles of the other species.

Since sexual isolation was the only isolating mechanism which could be readily measured, preliminary sexual isolation tests were made on the stocks at 16° C., using 10 females of each species and 10 males of either *D. pseudoobscura* or *D. persimilis*. The 30 flies used for each test were virgins and were kept together for a week in an ordinary shell vial with food, after which time the females were dissected and their seminal receptacles examined for sperm. The mating was done at 16° C. because at this temperature sexual isolation between *D. pseudoobscura* and *D. persimilis* is lowest (Mayr and Dobzhansky, 1945). For the population cage experiments, this temperature was desirable in order that changes in the sexual isolation could be most readily detected. For the later sexual isolation tests, the technique was similar, except

TABLE 1. *Main experiments, cage 2*

Generation	Number of parents		Number of offspring			Per cent hybrids
	pseudoobscura	*persimilis*	*pseudoobscura*	*persimilis*	hybrids	
1	320	680	82	313	227	36.5
2	60	60	561	473	322	23.7
3	300	300	246	995	665	34.9
4	120	120	406	336	126	14.5
5	250	250	162	86	292	54.1
6	80	80	273	415	38	5.2
7	180	180	617	478	17	1.5
10	150	150	820	257	72	6.3
11	180	180	653	729	42	2.9
12	440	440	2781	515	40	1.2
13	160	160	568	607	24	2.1
14	400	400	2217	712	259	8.1
15	300	300	2033	593	91	3.3
16	300	300	2613	453	89	2.8
17	300	300	2067	879	85	2.8
18	300	300	1925	1170	64	2.0
19	300	300	2808	1048	155	3.9
20	300	300	3372	1459	357	6.9
21	300	300	2109	1059	37	1.2
22	300	300	2117	1128	88	2.6

Equal numbers of males and females were used in all cases except in generation 6, in which 45 males and only 35 females of both *pseudoobscura* and *persimilis* were used.

TABLE 2. *Main experiments, cage 3*

Generation	Number of parents		Number of offspring			Per cent hybrids
	pseudoobscura	*persimilis*	*pseudoobscura*	*persimilis*	hybrids	
1	450	450	660	1008	464	22.5
2	360	360	544	1050	102	6.0
5	200	200	164	1680	100	5.1
6	130	130	394	1013	644	31.4
7	260	260	512	1569	58	2.7
8	360	360	648	1709	67	2.8
9	300	300	733	2193	60	2.0
10	300	300	1149	657	59	3.2
11	300	300	886	835	106	5.8
12	300	300	473	813	66	4.9
13	290	290	997	1965	159	5.1
14	300	300	1712	1712	182	5.0
15	300	300	1008	2418	79	2.2
16	300	300	1427	1772	180	5.3

TABLE 3. *Main experiments, cage 4*

Generation	Number of parents		Number of offspring			Per cent hybrids
	pseudoobscura	*persimilis*	*pseudoobscura*	*persimilis*	hybrids	
1	220	220	275	133	400	49.5
2	90	90	48	795	180	17.6
3	300	300	600	1006	55	3.3
4	400	400	414	1777	22	1.0
5	340	340	564	1241	25	1.4
6	300	300	1037	964	70	3.4
7	300	300	1214	244	26	1.8
8	150	150	275	1010	32	2.4
9	200	200	1284	1993	554	14.5
10	300	300	1375	3264	29	.6
11	300	300	1356	982	16	.7
12	300	300	2145	2188	74	1.7

that the males and females were aged apart for 10 days at 16° C., then left together for four hours before dissection.

The population cage experiments were done, also at 16° C., using the modified L'Hereditier-Teissier apparatus described by Dobzhansky (1947) and Wright and Dobzhansky (1946). In the present tests, however, the stender jars containing food with larvae were removed from the cages at the end of approximately two weeks and these cups fitted under sections of glass tubing two inches in diameter and approximately five inches in length, the tubes being fastened at the bottom with cellulose tape to the cup and closed at the top with a large cotton plug. The old cage with its adult flies was meanwhile discarded. In this way, all matings took place at 16° C., insuring a maximum number of hybrids, yet, by removing the covered cups to room temperature or to 25°C., more rapid development could take place. In addition, by not having long-continuous occupancy of the cages by the flies, the danger of infection with mites was greatly cut down. Flies were collected from the cups at 24-hour intervals, and counted according to whether they were *D. pseudoobscura, D. persimilis,* or hybrids. Advantage was taken of the fact that the size of the testis in hybrid males

is determined by which species was the mother and which the father, since the testis of hybrid males is large in the cross *pseudoobscura* female × *persimilis* male, but small in hybrids from the cross *persimilis* female × *pseudoobscura* male (Lancefield, 1929) (Dobzhansky, 1935). In this way, by observing the testis size, some idea could be gained of the way in which the isolating mechanisms were built up. The *D. pseudoobscura* and *D. persimilis* were then separated, the males separated from the females, the hybrids discarded, and all flies of pure species stored at 16° C., usually up to three weeks. Then equal numbers of males and females of both species were put into a fresh cage and the cycle recommenced. The number of flies put into the cage varied considerably according to the number of individuals of each sex and species that could be collected in three weeks (see Tables 1, 2, and 3), but usually was between 200 and 800 flies. The optimum for obtaining large numbers of all types of flies seemed to be about 600. The only modifications of this procedure were for certain generations of cage 2. When making up this cage for the first generation, more *persimilis* than *peudoobscura* were put in because it was thought at that time that the *pseudoobscura* would be more vigorous than the *persimilis*. When the cage was being made up for generation 6, only small numbers of *persimilis* were available, especially of *persimilis* females. Hence more males than females were put in, so that the effective population size would be as large as possible, while at the same time the numbers of the two pure species would be kept equal.

Late in the experiments, some mixed-cage tests were made. These were carried out as in the others, except that *D. persimilis* from one cage and *D. pseudoobscura* from another were used. These were run for one generation only.

Main Population Cage Experiments

Between ordinary laboratory *D. persimilis* and *D. pseudoobscura,* several isolating mechanisms can be observed in operation (Dobzhansky and Epling, 1944). First of all, there is usually considerable sexual isolation, varying with the stocks used and with the temperature (Mayr and Dobzhansky, 1945). Hybrids seem to have the same viability as the pure species, but hybrid males are completely sterile. Females, when backcrossed to either parental species, lay the usual number of eggs, but the larvae arising from these eggs have such poor viability that in competition with larvae of the pure species, as in population cages, they never reach the adult stage. Hence, in a population cage with both species present, even if the hybrids were not removed each

Table 4. *Results of partial correlation tests*

	S	σ_S	p	Correlation (r)
Hybrids × total flies (2, 3)				
Cage 2	−70	30.8	.05 > but > .01	−.368
Cage 3	−21	18.3	> .05	−.231
Cage 4	−34	14.6	.05 > but > .01	−.515
Total flies × time (1, 2)				
Cage 2	122	30.8	< .01	.642
Cage 3	33	18.3	> .05	.363
Cage 4	38	14.6	< .01	.576
Hybrids × time (1, 3)				
Cage 2	−140	30.8	< .01	−.737
Cage 3	−19	18.3	> .05	−.209
Cage 4	−26	14.6	> .05	−.394

generation, as was done in the present experiments, no introgressive hybridization would be expected to occur. That such introgression is indeed lacking was established by Dobzhansky (1945).

Preliminary sexual isolation tests (see table 4) showed, for the original stocks, high sexual isolation using *pseudoobscura* males, but little or no sexual isolation using *persimilis males*. After outcrossing to wild strains and reextracting, the sexual isolation with *pseudoobscura* males was lowered somewhat, but with *persimilis* males was somewhat raised. This rise in isolation index is, it should be noted, non-significant, essentially random mating still taking place when *persimilis* males are used. Hence, in a cage containing both species, a considerable number of hybrids would be expected in the first generation. This is borne out by the first generation counts from the three population cages which could be carried to completion, the percentages being 50, 36, and 22. In earlier experiments, using other stocks, from population cages which had to be discarded early because of mite infection, the hybrid percentages for the first generations were 60 and 27. In all population cages, however, which ran for more than four generations, a rather rapid decrease in the number of hybrids occurred. (See figs. 1–3 and tables 1, 2, and 3.) In all cases, within six generations, the percentage of hybrids had fallen to 5 per cent (though in some cases with some later temporary increase), and in certain cases reached as low as 1 per cent in later generations. This is in full agreement with Dobzhansky's work (1945), in which the percentage of hybrids in a population cage kept at 16° C. fell from 24 per cent on February 3 to 3.6 per cent on June 11.

A considerable heterogeneity may be noted in the percentage of hybrids after the first few generations; it may also be noted that in later generations a larger total number of flies was collected than earlier. It was therefore advisable to test the heterogeneity for significance and to determine whether the decrease in the percentage of hybrids could be explained by a correlation of percentages of hybrids

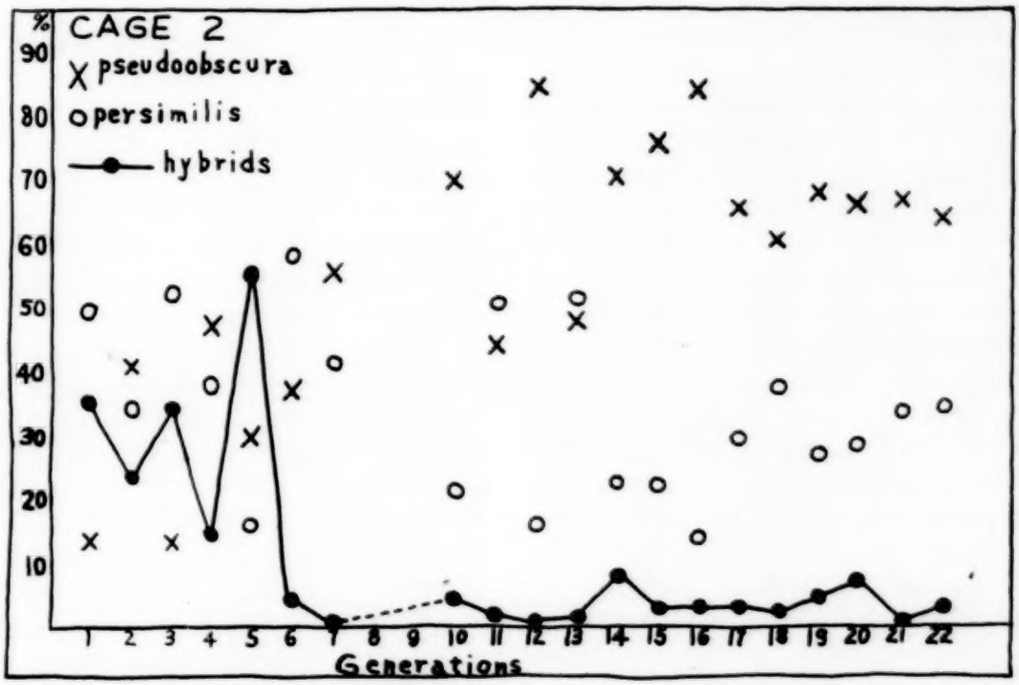

FIG. 1. Change in frequency of hybrids in cage 2, showing the percentage of the two pure species and the hybrids in each generation. (For additional data, see table 1.) No data for generations 8 and 9.

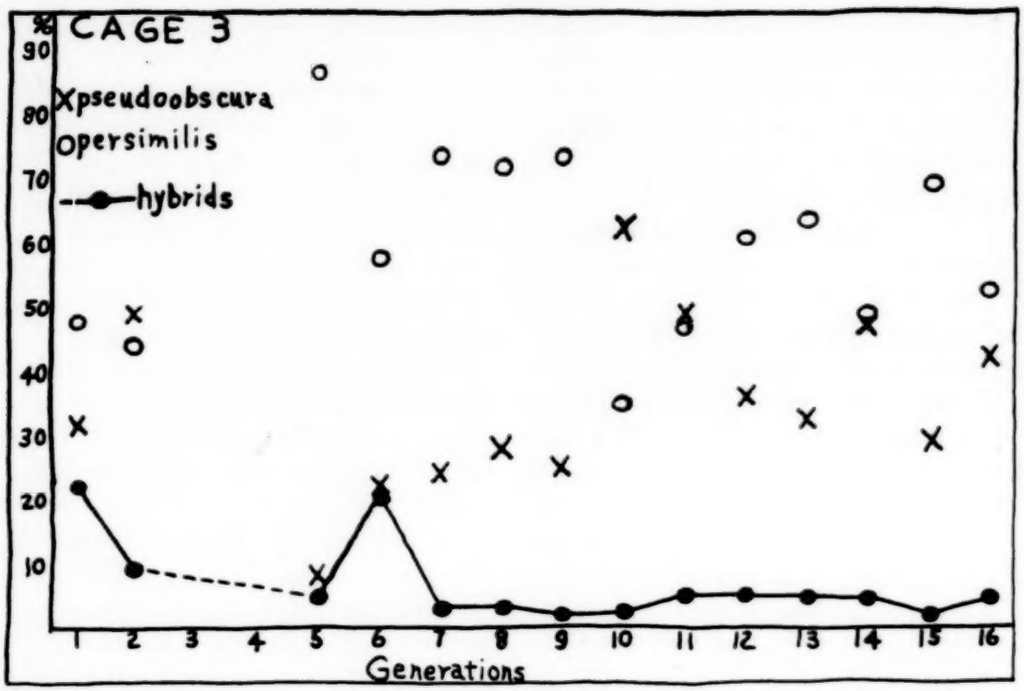

FIG. 2. Change in frequency of hybrids in cage 3, showing the percentage of the two pure species and their hybrids in each generation. (For additional data, see table 2.) No data for generations 3 and 4.

with the total numbers of flies. Such a correlation was possible, since the numbers of flies introduced each time the cage was reloaded were approximately the same, especially in later generations, and certainly showed no particular trend. The increase in total numbers of flies collected from generation to generation, therefore, was due to an increase in the percentage of eggs laid which later developed into adult flies. This meant a decrease in selection pressure for viability factors. Since the hybrids, being the only wild-type flies, were known to be superior in viability, reduced selection decreased their advantage over the mutant pure species. Hence their frequency would be reduced.

To test the heterogeneity, a χ^2 test was made for each cage, using the numbers of hybrids and pure species for each generation after a low value for the hybrids had been reached. The test, therefore involved generations 6, 7, and 10–22 for cage 2, generations 7–16 for cage 3, and generations 3–12 for cage 4. The χ^2 obtained was very high for all three cages, 550 for cage 2, 138 for cage 3, and 1,680 for cage 4. The exceedingly high χ^2 for cage 4 was chiefly caused by the very large number of hybrids in generation 9. All three χ^2 values are highly significant, with a probability far below .01. It is evident, therefore, that even after the early generations, the frequency of hybrids was undergoing significant changes. The causes of these changes are, however, not clear.

To determine how the percentage of hybrids was correlated with time and with total number of flies per generation, rank correlation tests were performed. Three of these tests were made for each cage, percentage hybrids × time, percentage hybrids × total flies, and total flies × time. Time is considered variable 1, total flies as variable 2, and percentage hybrids as variable 3. The method used was that of Kendall (1943). The results may be seen in table 4.

It is evident that the conditions in the three cages are somewhat different. In cage 2, the percentage of hybrids shows a very significant decrease, and the total number of flies a significant increase, but

the correlation of number of flies and hybrids is only on the borderline of significance. In cage 3, all three correlations are non-significant. In cage 4, conditions are essentially as in cage 2 except that the correlation of total flies × time is higher than the correlation of hybrids × time in cage 4, but not in cage 2.

In order to determine the partial correlation between percentage of hybrids and total, at the same time correcting for correlation of both with time, the formula

$$r_{23.1} = \frac{r_{23} - r_{13}\, r_{12}}{\sqrt{(1 - r_{12}^2)\,(1 - r_{13}^2)}}$$

(Kendall, 1942) was used. (Kendall gives no method for testing the significance of this partial rank correlation.) This formula gave a value of .20 for cage 2, − .17 for cage 3, and − .38 for cage 4. This shows that the partial correlation between total flies and percentage of hybrids may be either positive or negative and in two of the three cages is rather small. It therefore seems improbable that the correlation of percentage of hybrids with time can be explained by the correlation of total flies with time and of total flies with percentage of hybrids. It must also be remembered that these correlations apply to the entire period during which flies were being kept in each cage. It was only during the early generations, however, with a very few exceptions, that the percentage of hybrids was undergoing much change. The rank correlation test used disregards the magnitude of the changes and reflects only the rank of the values for total flies, per cent hybrids, and time. A real decrease in frequency of hybrids formed seems indicated. Natural selection would appear to be building up new reproductive isolation.

The detailed history of the three cages, of course, differed considerably. Cage 4 showed a very rapid steady drop in the percentage of hybrids, whereas cages 2 and 3 took a longer time and showed considerable fluctuations. In the two earlier cages, the percentage of hybrids for certain generations is unknown; generations 8 and 9 for cage 2, generations 3 and 4 for cage 3. This unfortunate state of affairs arose from widespread contamina-

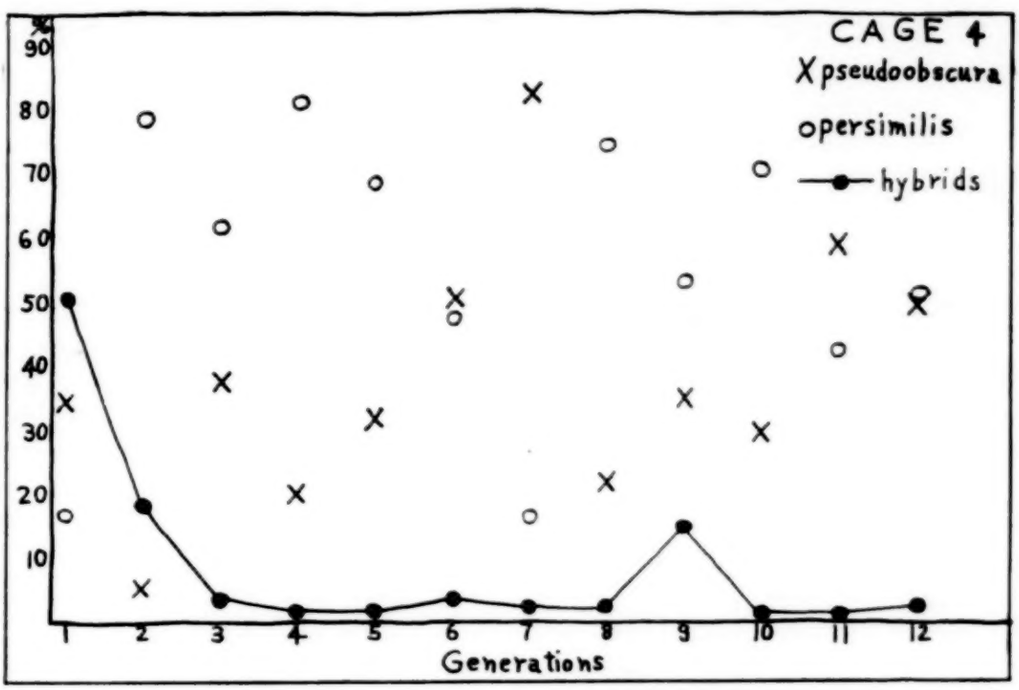

FIG. 3. Change in frequency of hybrids in cage 4, showing the percentage of the two pure species and their hybrids in each generation. (For additional data, see table 3.)

TABLE 5. *Source of hybrid males*

Generation	Cage 2		Cage 3		Cage 4	
	per. ♂ × *pseudo.* ♀	*pseudo.* ♂ × *per.* ♀	*per.* ♂ × *pseudo.* ♀	*pseudo.* ♂ × *per.* ♀	*per.* ♂ × *pseudo.* ♀	*pseudo.* ♂ × *per.* ♀
1	?	?	189	10	136	66
2	122	21	37	29	48	21
3	192	10	?	?	4	21
4	52	0	?	?	1	8
5	92	6	3	60	8	4
6	20	1	0	103	14	16
7	?	?	1	39	8	5
8	?	?	9	26	6	4
9	?	?	11	29	169	103
10	12	20	16	7	2	16
11	17	3	44	5	0	8
12	15	1	21	8	3	35
13	4	3	7	88		
14	84	14	45	37		
15	11	34	16	18		
16	42	17	72	6		
17	17	13				

tion of both cages by wild type *D. pseudoobscura* or *D. persimilis* which occurred at this time. Even after the percentage of hybrids had reached a low level, there was a certain amount of fluctuation, the extreme being in cage 4, where the frequency of hybrids rose from 2 per cent in generation 8 to 14 per cent in generation 9 and back to 1 per cent in generation 10, thereafter remaining at a low level. The causes of these earlier and later fluctuations are unknown. They could not be caused by contamination since this was ruled out by the examination of wild type male testes. These dissections showed no sharp rises in percentage of flies with large testes corresponding to high frequencies of wild type flies. (Compare tables 1, 2, and 3 with table 5.) This would have been the case if any appreciable contamination had occurred at these times. Indeed, in those generations in which data are lacking because widespread contamination was known to occur, almost all wild type males examined had full-sized testes. It might be argued that some of the later rise might be due to the contamination earlier, resulting in the introduction of unselected genes, thereby reducing the reproductive isolation in the population. This, however, could only occur if appreciable numbers of the contaminants were *glass pseudoobscura* or *orange persimilis*. Otherwise, the F1 flies from the interbreeding of cage and contaminating flies would be wild type and would be discarded along with the species hybrids. The effects of contamination could therefore be confined to the generation immediately following the contamination. Reasons for the fluctuations must therefore be sought from other causes. Temperature was usually fairly constant, especially during the latter part of the experiments, but humidity was usually quite variable, and may have differentially affected the viability of the wild type hybrids, and even of the degree of reproductive isolation itself. These earlier and later fluctuations, however, do not in any way vitiate the clear demonstration that hybrids are in general much less frequent for the later generations than for the earlier ones in all three cages.

Another fluctuating variable was the relative number of pure *pseudoobscura* and *perisimilis* in each generation. Differences between generations, even in the

same cage, were often extreme. The relative numbers of the two species might even undergo a radical shift in one generation. This may be seen by comparing the counts of generations 7 and 8 in cage 4. These fluctuations in the number of pure species flies also point to considerable environmental fluctuations, which caused natural selection to favor now one, now the other species.

From the examination of the hybrid male testes, an interesting fact emerges (see table 5). In the first two generations, the number of hybrids coming from the cross *persimilis* male × *pseudoobscura* female outnumbered and usually greatly outnumbered those from the cross of *pseudoobscura* male × *persimilis* female. This is to be expected, since, in the preliminary sexual isolation tests, *pseudoobscura* males already showed a strong preference for their own females, whereas the *persimilis* males showed little or no such preference. In the later generations, however, the hybrids seemed to be much more randomly distributed between the two types, often varying rather widely among cages and from generation to generation within the same cage, now one, now the other kind of hybrid being more numerous. This was, to some extent, due to the small number of hybrid males which could usually be dissected in these later generations. In general, therefore, natural selection has acted mainly to reduce the number of fertilizations of *pseudoobscura* females by *persimilis* males, making their number roughly comparable to those of the reciprocal cross.

Multiple-Choice Experiments

When cage 2 had run for 10 generations and cage 3 for 5 generations, flies from each were subcultured in bottles and standard sexual isolation tests were made, using as a control the stocks from which the cages were originally made up. When the males were *persimilis*, the isolation index was not significantly different from zero when the original control stocks were used, but it was highly significant when the flies from cages 2 and 3 were used. As would be expected, the value of the isolation index for cage 2 (.52) was greater than the value (.30) for cage 3 (see table 6).

When cage 2 had run for 14 generations, cage 3 for 9 generations, and cage

Table 6. *Sexual isolation tests*

Strains tested	No. of ♀ ♀ inseminated and not inseminated				Isolation index*	χ^2	Probability
	Homogamic		Heterogamic				
	+	−	+	−			
persimilis ♂ ♂—Preliminary test 1	46	37	55	36	−.04	.7	.4
persimilis ♂ ♂—Preliminary test 2	31	17	30	29	.12	1.5	.2
persimilis ♂ ♂—Control 1	82	130	80	125	−.07	.02	.9
persimilis ♂ ♂—Cage 2, 10th generation	129	66	40	152	.52	98.68	<.01
persimilis ♂ ♂—Cage 3, 5th generation	99	84	54	132	.30	22.77	<.01
persimilis ♂ ♂—Control 2	107	71	68	108	.21	15.48	<.01
persimilis ♂ ♂—Cage 2, 14th generation	97	19	25	91	.60	87.15	<.01
persimilis ♂ ♂—Cage 3, 9th generation	75	15	23	66	.52	57.01	<.01
persimilis ♂ ♂—Cage 4, 5th generation	106	41	26	128	.62	89.74	<.01
pseudoobscura ♂ ♂—Preliminary test 1	81	14	3	86	.93	120.9	<.01
pseudoobscura ♂ ♂—Preliminary test 2	42	13	6	45	.73	42.0	<.01
pseudoobscura ♂ ♂—Control 1	75	32	11	88	.73	71.16	<.01
pseudoobscura ♂ ♂—Cage 2, 10th generation	120	9	2	126	.96	211.9	<.01
pseudoobscura ♂ ♂—Cage 3, 5th generation	87	11	6	92	.87	131.0	<.01
pseudoobscura ♂ ♂—Control 2	59	6	17	50	.57	55.12	<.01
pseudobscura ♂ ♂—Cage 2, 14th generation	60	7	7	60	.80	79.53	<.01
pseudoobscura ♂ ♂—Cage 3, 9th generation	75	3	21	59	.57	78.55	<.01
pseudoobscura ♂ ♂—Cage 4, 5th generation	57	9	4	62	.87	77.68	<.01

* The isolation index is calculated from the percentage of homo- and heterogamic females inseminated (see Dobzhansky and Mayr, 1944), + 1.00 indicating only homogamic mating, 0.00 random mating, and −1.00 completely heterogamic mating.

is determined by which species was the mother and which the father, since the testis of hybrid males is large in the cross *pseudoobscura* female × *persimilis* male, but small in hybrids from the cross *persimilis* female × *pseudoobscura* male (Lancefield, 1929) (Dobzhansky, 1935). In this way, by observing the testis size, some idea could be gained of the way in which the isolating mechanisms were built up. The *D. pseudoobscura* and *D. persimilis* were then separated, the males separated from the females, the hybrids discarded, and all flies of pure species stored at 16° C., usually up to three weeks. Then equal numbers of males and females of both species were put into a fresh cage and the cycle recommenced. The number of flies put into the cage varied considerably according to the number of individuals of each sex and species that could be collected in three weeks (see Tables 1, 2, and 3), but usually was between 200 and 800 flies. The optimum for obtaining large numbers of all types of flies seemed to be about 600. The only modifications of this procedure were for certain generations of cage 2. When making up this cage for the first generation, more *persimilis* than *pseudoobscura* were put in because it was thought at that time that the *pseudoobscura* would be more vigorous than the *persimilis*. When the cage was being made up for generation 6, only small numbers of *persimilis* were available, especially of *persimilis* females. Hence more males than females were put in, so that the effective population size would be as large as possible, while at the same time the numbers of the two pure species would be kept equal.

Late in the experiments, some mixed-cage tests were made. These were carried out as in the others, except that *D. persimilis* from one cage and *D. pseudoobscura* from another were used. These were run for one generation only.

Main Population Cage Experiments

Between ordinary laboratory *D. persimilis* and *D. pseudoobscura*, several isolating mechanisms can be observed in operation (Dobzhansky and Epling, 1944). First of all, there is usually considerable sexual isolation, varying with the stocks used and with the temperature (Mayr and Dobzhansky, 1945). Hybrids seem to have the same viability as the pure species, but hybrid males are completely sterile. Females, when backcrossed to either parental species, lay the usual number of eggs, but the larvae arising from these eggs have such poor viability that in competition with larvae of the pure species, as in population cages, they never reach the adult stage. Hence, in a population cage with both species present, even if the hybrids were not removed each

Table 4. *Results of partial correlation tests*

	S	σ_S	p	Correlation (r)
Hybrids × total flies (2, 3)				
Cage 2	−70	30.8	.05>but>.01	−.368
Cage 3	−21	18.3	>.05	−.231
Cage 4	−34	14.6	.05>but>.01	−.515
Total flies × time (1, 2)				
Cage 2	122	30.8	<.01	.642
Cage 3	33	18.3	>.05	.363
Cage 4	38	14.6	<.01	.576
Hybrids × time (1, 3)				
Cage 2	−140	30.8	<.01	−.737
Cage 3	−19	18.3	>.05	−.209
Cage 4	−26	14.6	>.05	−.394

generation, as was done in the present experiments, no introgressive hybridization would be expected to occur. That such introgression is indeed lacking was established by Dobzhansky (1945).

Preliminary sexual isolation tests (see table 4) showed, for the original stocks, high sexual isolation using *pseudoobscura* males, but little or no sexual isolation using *persimilis males*. After outcrossing to wild strains and reextracting, the sexual isolation with *pseudoobscura* males was lowered somewhat, but with *persimilis* males was somewhat raised. This rise in isolation index is, it should be noted, non-significant, essentially random mating still taking place when *persimilis* males are used. Hence, in a cage containing both species, a considerable number of hybrids would be expected in the first generation. This is borne out by the first generation counts from the three population cages which could be carried to completion, the percentages being 50, 36, and 22. In earlier experiments, using other stocks, from population cages which had to be discarded early because of mite infection, the hybrid percentages for the first generations were 60 and 27. In all population cages, however, which ran for more than four generations, a rather rapid decrease in the number of hybrids occurred. (See figs. 1–3 and tables 1, 2, and 3.) In all cases, within six generations, the percentage of hybrids had fallen to 5 per cent (though in some cases with some later temporary increase), and in certain cases reached as low as 1 per cent in later generations. This is in full agreement with Dobzhansky's work (1945), in which the percentage of hybrids in a population cage kept at 16° C. fell from 24 per cent on February 3 to 3.6 per cent on June 11.

A considerable heterogeneity may be noted in the percentage of hybrids after the first few generations; it may also be noted that in later generations a larger total number of flies was collected than earlier. It was therefore advisable to test the heterogeneity for significance and to determine whether the decrease in the percentage of hybrids could be explained by a correlation of percentages of hybrids

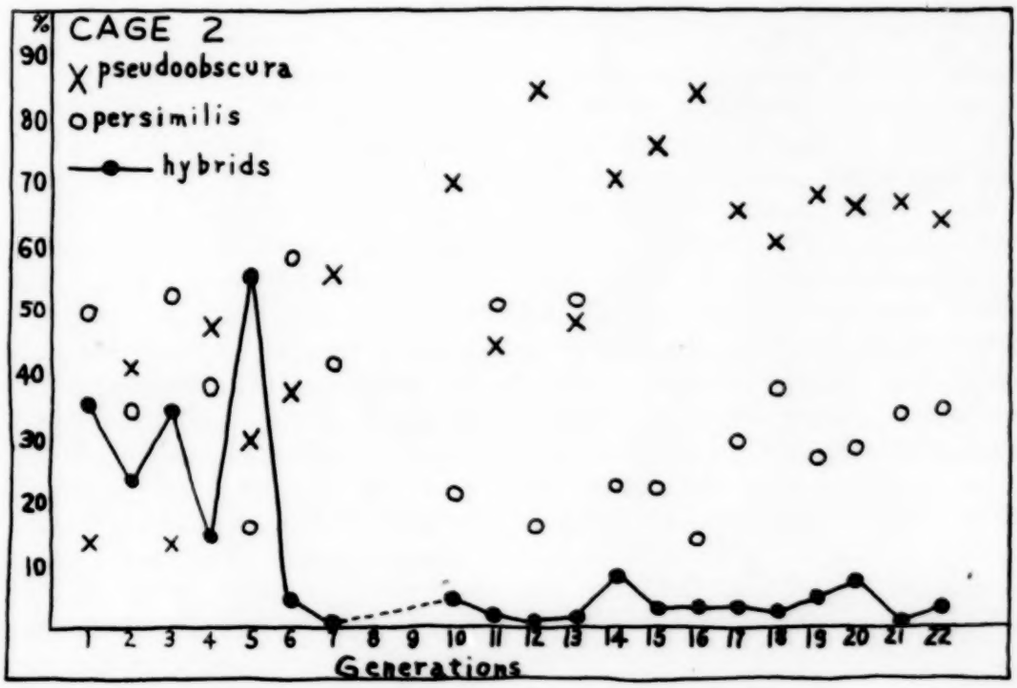

FIG. 1. Change in frequency of hybrids in cage 2, showing the percentage of the two pure species and the hybrids in each generation. (For additional data, see table 1.) No data for generations 8 and 9.

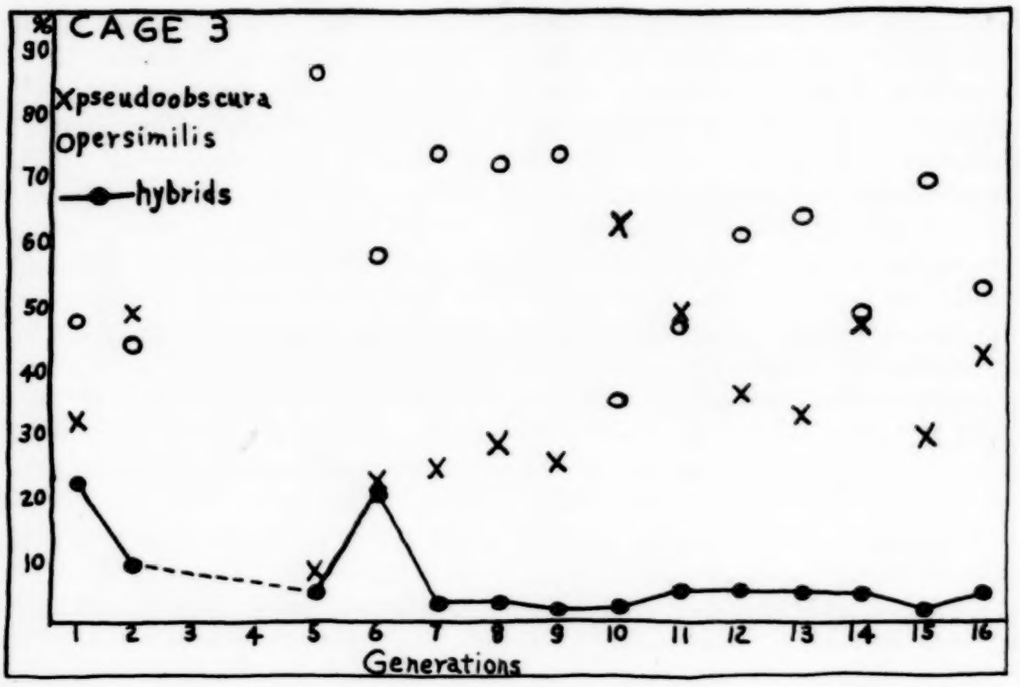

FIG. 2. Change in frequency of hybrids in cage 3, showing the percentage of the two pure species and their hybrids in each generation. (For additional data, see table 2.) No data for generations 3 and 4.

with the total numbers of flies. Such a correlation was possible, since the numbers of flies introduced each time the cage was reloaded were approximately the same, especially in later generations, and certainly showed no particular trend. The increase in total numbers of flies collected from generation to generation, therefore, was due to an increase in the percentage of eggs laid which later developed into adult flies. This meant a decrease in selection pressure for viability factors. Since the hybrids, being the only wild-type flies, were known to be superior in viability, reduced selection decreased their advantage over the mutant pure species. Hence their frequency would be reduced.

To test the heterogeneity, a χ^2 test was made for each cage, using the numbers of hybrids and pure species for each generation after a low value for the hybrids had been reached. The test, therefore involved generations 6, 7, and 10–22 for cage 2, generations 7–16 for cage 3, and generations 3–12 for cage 4. The χ^2 obtained was very high for all three cages, 550 for cage 2, 138 for cage 3, and 1,680 for cage 4. The exceedingly high χ^2 for cage 4 was chiefly caused by the very large number of hybrids in generation 9. All three χ^2 values are highly significant, with a probability far below .01. It is evident, therefore, that even after the early generations, the frequency of hybrids was undergoing significant changes. The causes of these changes are, however, not clear.

To determine how the percentage of hybrids was correlated with time and with total number of flies per generation, rank correlation tests were performed. Three of these tests were made for each cage, percentage hybrids × time, percentage hybrids × total flies, and total flies × time. Time is considered variable 1, total flies as variable 2, and percentage hybrids as variable 3. The method used was that of Kendall (1943). The results may be seen in table 4.

It is evident that the conditions in the three cages are somewhat different. In cage 2, the percentage of hybrids shows a very significant decrease, and the total number of flies a significant increase, but

the correlation of number of flies and hybrids is only on the borderline of significance. In cage 3, all three correlations are non-significant. In cage 4, conditions are essentially as in cage 2 except that the correlation of total flies × time is higher than the correlation of hybrids × time in cage 4, but not in cage 2.

In order to determine the partial correlation between percentage of hybrids and total, at the same time correcting for correlation of both with time, the formula

$$r_{23.1} = \frac{r_{23} - r_{13}\, r_{12}}{\sqrt{(1 - r_{12}^2)\,(1 - r_{13}^2)}}$$

(Kendall, 1942) was used. (Kendall gives no method for testing the significance of this partial rank correlation.) This formula gave a value of .20 for cage 2, − .17 for cage 3, and − .38 for cage 4. This shows that the partial correlation between total flies and percentage of hybrids may be either positive or negative and in two of the three cages is rather small. It therefore seems improbable that the correlation of percentage of hybrids with time can be explained by the correlation of total flies with time and of total flies with percentage of hybrids. It must also be remembered that these correlations apply to the entire period during which flies were being kept in each cage. It was only during the early generations, however, with a very few exceptions, that the percentage of hybrids was undergoing much change. The rank correlation test used disregards the magnitude of the changes and reflects only the rank of the values for total flies, per cent hybrids, and time. A real decrease in frequency of hybrids formed seems indicated. Natural selection would appear to be building up new reproductive isolation.

The detailed history of the three cages, of course, differed considerably. Cage 4 showed a very rapid steady drop in the percentage of hybrids, whereas cages 2 and 3 took a longer time and showed considerable fluctuations. In the two earlier cages, the percentage of hybrids for certain generations is unknown; generations 8 and 9 for cage 2, generations 3 and 4 for cage 3. This unfortunate state of affairs arose from widespread contamina-

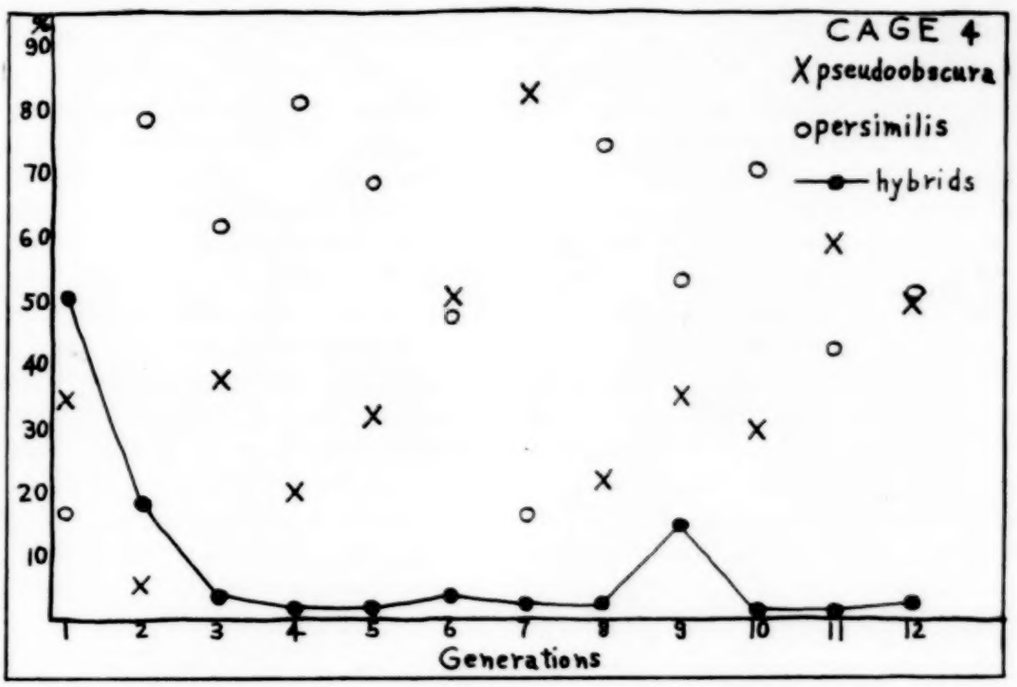

FIG. 3. Change in frequency of hybrids in cage 4, showing the percentage of the two pure species and their hybrids in each generation. (For additional data, see table 3.)

TABLE 5. *Source of hybrid males*

Generation	Cage 2		Cage 3		Cage 4	
	per. ♂ × *pseudo.* ♀	*pseudo.* ♂ × *per.* ♀	*per.* ♂ × *pseudo.* ♀	*pseudo.* ♂ × *per.* ♀	*per.* ♂ × *pseudo.* ♀	*pseudo.* ♂ × *per.* ♀
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13	4	3	7	88		
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same cage, were often extreme. The relative numbers of the two species might even undergo a radical shift in one generation. This may be seen by comparing the counts of generations 7 and 8 in cage 4. These fluctuations in the number of pure species flies also point to considerable environmental fluctuations, which caused natural selection to favor now one, now the other species.

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Multiple-Choice Experiments

When cage 2 had run for 10 generations and cage 3 for 5 generations, flies from each were subcultured in bottles and standard sexual isolation tests were made, using as a control the stocks from which the cages were originally made up. When the males were *persimilis*, the isolation index was not significantly different from zero when the original control stocks were used, but it was highly significant when the flies from cages 2 and 3 were used. As would be expected, the value of the isolation index for cage 2 (.52) was greater than the value (.30) for cage 3 (see table 6).

When cage 2 had run for 14 generations, cage 3 for 9 generations, and cage

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pseudoobscura ♂ ♂—Preliminary test 2	42	13	6	45	.73	42.0	<.01
pseudoobscura ♂ ♂—Control 1	75	32	11	88	.73	71.16	<.01
pseudoobscura ♂ ♂—Cage 2, 10th generation	120	9	2	126	.96	211.9	<.01
pseudoobscura ♂ ♂—Cage 3, 5th generation	87	11	6	92	.87	131.0	<.01
pseudoobscura ♂ ♂—Control 2	59	6	17	50	.57	55.12	<.01
pseudobscura ♂ ♂—Cage 2, 14th generation	60	7	7	60	.80	79.53	<.01
pseudoobscura ♂ ♂—Cage 3, 9th generation	75	3	21	59	.57	78.55	<.01
pseudoobscura ♂ ♂—Cage 4, 5th generation	57	9	4	62	.87	77.68	<.01

* The isolation index is calculated from the percentage of homo- and heterogamic females inseminated (see Dobzhansky and Mayr, 1944), + 1.00 indicating only homogamic mating, 0.00 random mating, and −1.00 completely heterogamic mating.

4 for 5 generations, a second series of sexual isolation tests were performed, using the same technique and again with the original stocks subcultured generation after generation as controls. In this second set of tests, using *persimilis* males, the controls as well as the experimental cultures gave a value highly significantly different from random mating, though the isolation indices were much lower than those for the flies from the cages (.21 as opposed to .60, .52, and .62). In order to determine whether the sexual isolation for the flies taken from the cages was significantly different from those of the controls, the procedure used by Snedecor (1946) for three sets of attributes (Section 9.8) was applied. Due to the extremely laborious nature of the procedure, the comparison was made only between the control and the cage test most like the control in χ^2 and isolation index, namely cage 3. The χ^2 for these two sets of tests was highly significant ($\chi^2 = 15.81$, probability $< .01$ for 1 degree of freedom). Since the other cages had each given a χ^2 and isolation index even more different from that of the controls, it was evident that for the second set of tests, as well as for the first, the flies from cages which had run for several generations displayed a definitely greater interspecific sexual isolation than did the controls. As would be expected, both control and experimental tests using *pseudoobscura* males showed strong sexual isolation (see table 4). From these results, it would certainly appear that at least part of the reproductive isolation observed in the cages is sexual.

In this connection, it was thought that certain comparisons of sexual isolation between different cages might prove interesting using *persimilis* males, of course. This could also be done using the procedure for three sets of attributes from Snedecor. The first such comparison was made between the sexual isolation in cage 2 generation 10 and cage 3 generation 5. Both of these series of tests come from the first set. At the time the flies used in making up the stocks for this set of tests were taken from the cages, the percentage of hybrids in cage 2 stood at 6 per cent and had probably stayed at this point or lower for five generations. Never afterwards was the percentage of hybrids in this cage to go above 8 per cent. At the same time, however, cage 3, though it stood at 5 per cent, had probably never previously had such a small percentage of hybrids, and the fact that in the next generation it rose again to 21 per cent shows that the isolating mechanisms had not yet been stabilized. When the tests from these two cages, both from the first set, are compared statistically, we obtain a χ^2 of 7.67, which for one degree of freedom gives a probability of $< .01$.

The second comparison was made of the sexual isolation for cage 3 between generation 5 and generation 9. At generation 5, the isolating mechanisms had not yet been stabilized, as has already been mentioned, but by generation 9, the percentage of hybrids stood at 2 per cent and was never afterward to rise above 6 per cent. Hence, a difference might be expected. This comparison was, of course, not as valid as that between cage 2 and cage 3 from the first set of tests, since the two series of tests in this case were not done during the same period, and hence possibly not under strictly comparable conditions. Nevertheless, the results of this comparison might at least be suggestive. In this case, a χ^2 was obtained of 9.83, with a probability for one degree of freedom of $< .01$. These two comparisons would seem to give support to the idea that during the earlier generations, sexual isolation was increasing in all cages.

Mixed-Cage Experiments

In order to determine whether the isolation observed in the cages acts in general against all members of the other species, or is a more specific mechanism, isolating only the strains from the cage in which it is developed, mixed cage experiments were undertaken. In these

TABLE 7. *Mixed cage experiments*

(Equal numbers of each sex and species used as parents in each case)

Source of parents		Number of offspring			Per cent hybrids
pseudoobscura	*persimilis*	*pseudoobscura*	*persimilis*	hybrids	
Cage 3—10th generation	Cage 4—6th generation	889	840	85	5
Cage 4—6th generation	Cage 3—10th generation	314	1436	95	5
Cage 2—19th generation	Cage 4—9th generation	1143	731	270	13
Cage 4—9th generation	Cage 2—19th generation	1186	818	39	2
Cage 2—19th generation	Cage 3—13th generation	2535	873	117	3
Cage 3—15th generation	Cage 2—21st generation	838	1091	64	3

tests, *peudoobscura* and *persimilis* were taken from different cages, each of which had run for several generations with a very low percentage of hybrids, and were put together in one cage, which was permitted to proceed for one generation and then the number of pure species and hybred flies were counted. All combinations of the three cages were made. The results may be seen in table 7. In all cases but one, the percentages of the hybrids were quite low and approximately the same as those appearing in the unmixed cages. The only exception was when *pseudoobscura* from cage 2 were put together from cage 4. The percentage of hybrids obtained in this case, while not quite as high as one later count from one of the unmixed cages (cage 4 generation 9), was considerably higher than was usual in these later counts. On the other hand, it was a good deal lower than even the lowest of the first generation counts from the unmixed cages (13 per cent as compared with 22 per cent). From this it appears that these isolating mechanisms selected out in each of the three cages were very similar, so much so that they could be readily interchanged and probably were mechanisms which would react towards all individuals of the other species or at least that segment sampled by the original stocks, rather than with only the particular strain against which they had been developed. This was perhaps to be expected, inasmuch as all three cages had been made up from the same stocks, so that all initially carried the same store of genetic variability for reproductive isolation, except for differences due to the sampling error involved in making up the cages. Under these circumstances, one might expect natural selection to fix essentialy the same gene complexes in each cage. An alternative hypothesis would be, of course, that different gene complexes having very similar phenotypic effects, or, at least, all acting effectively-against stock flies of the other species as a whole, were selected out in each cage. To me, at least, this would appear to be much less likely.

DISCUSSION AND CONCLUSIONS

Before attempting to evaluate these findings, it may be desirable to review briefly the effective isolating mechanisms between *Drosophila pseudoobscura* and *D. persimilis* existing in nature. Geographical isolation can hardly be said to exist, since the range of *D. persimilis* is entirely included in that of *D. pseudoobscura*. Ecological isolation, on the other hand, is considerable and is twofold. The two species have rather different macro-ecological or ecoclimatic preferences, *D. persimilis* being found, for the most part, at higher elevations in the mountains, *D. pseudoobscura*, to a greater extent, at lower elevations, including the lowlands (Dobzhansky and Epling, 1944). It is interesting to note that outside the range of *D. persimilis*, *D. pseudoobscura* is common in the mountains as well as in the lowlands, reaching the tree line, above 11,000 feet, on Pikes peak in Colorado.

Micro-ecological or ecotopic differences are also marked, *D. pseudoobscura* preferring warmer sunnier places. Whereas *D. persimilis* prefers the cooler shadier spots (Pittendrigh-unpublished). Sexual isolation seems to play an important part, newly captured strains having a rather high sexual isolation except at low temperatures. All these isolating mechanisms, however, are partial, and one would expect some hybrids to be formed in nature. This apparently does not occur, since of the thousands of salivary gland chromosomes which have been studied from flies caught in nature, not a single hybrid has been found, even from localities where both species occur together (Dobzhansky-unpublished). This total absence of hybrids is at present an unsolved problem. It may be due to other isolating mechanisms, not yet detected, which acting in conjunction with the known ones, completely prevent the appearance of hybrids. Those two isolating mechanisms, male hybrid sterility and backcross inviability, which are so important in keeping the species apart in the laboratory, apparently never have a chance to operate in nature, though of course they may have been important in preventing gene exchange in the past.

With these facts in mind, let us consider the central problem of whether natural selection can act to build up isolating mechanisms between distinct allopatric forms whose ranges meet. In the first place, there must already be some isolation before selection can act, both in a form which makes the hybrid less well adapted, and also as hereditary variability for further isolation. If the hybrid is as well adapted as either parental type for any availible environment, either parental or intermediate, the hybrids will not be discriminated against and the two original forms will be connected by a zone of subspecific intergradation. Hence it means that the first isolating mechanisms must arise as by-products of genetic divergence, but at least theoretically could be added to and built into a "gene-tight" isolating system by selection.

Up until now, however, there has been no direct evidence that selection can and does do this. To the knowledge of the writer, no experimental evidence other than that here cited has been brought forward to show that natural selection can create or strengthen an isolating mechanism. Direct evidence of this occurrence from natural populations is also very scanty and often capable of interpretation on other grounds. Perhaps the best established case concerns sexual isolation between *D. pseudoobscura* and *D. miranda* (Dobzhansky and Koller, 1939). Here, strains of *D. pseudoobscura* coming from localities within or close to the range of at least the northern populations of *D. miranda* show in general a greater sexual isolation with northern strains of *D. miranda* than do strains of *D. pseudoobscura* coming from more distant localities. It is probable that in populations of *D. pseudoobscura* living in close proximity to *D. miranda*, natural selection has acted to prevent hybridization by means of greater sexual isolation, whereas in populations which are geographically remote from the range of *D. miranda*, increased sexual isolation has had no such selective advantage. However, even here the situation is not so simple, since this relation fails to hold when southern rather than northern strains of *D. miranda* are tested against the geographical strains of *D. pseudoobscura*.

A more disputed situation occurs in the cases of the crows, *Corvus corone-cornix*, and the grackles, *Quiscalus quiscula-aeneus* discussed by Dobzhansky (1941). In both these cases, the hybrid zone between the ranges of the two incipient species is broad in northern regions in which the retreat of the glaciers has only recently permitted occupation by the parental forms, whereas the hybrid zone is narrow in the south where the two forms in each case have been able to occupy the habitat for a longer time. Dobzhansky interprets the facts as indicating

a spread, with retreat of the glaciers, from geographically isolated refuges in the far south. As the two forms spread north, they came in contact first in the more southern regions, later in the north. In the south, natural selection has been able to build up reproductive isolation, preventing much hybridization, whereas in the north, not enough time has elaspsed for this to occur. This hypothesis has not been favored by Mayr (1942), who attributes the narrow hybrid zone in the more southern localities to local ecological factors preventing the two forms from coming in contact to as great a degree as in the north. It now seems probable, however, that an extensive study of cases where incipient species have come together, in different places, at different times, after being geographically isolated, would reveal undoubted cases of natural selection for reproductive isolation.

The evidence here presented shows, not only that natural selection can act to strengthen isolation between species, but it also brings out an important aspect of the difference between species and subspecies. When subspecies are involved, gene exchange between the two populations is always possible, the hybrid types are fitted for various intermediate habitats, geographical or ecological, between those of the two parental types, and fit into their population structure. Once the threshold has been passed, however, and the two populations have reached the status of full species, the relation between them becomes quite different. The hybrids are relatively ill-adapted for any available habitat. There are usually several isolating mechanisms which to a greater or lesser degree prevent gene exchange, and these are likely to be strengthened and added to by natural selection. Furthermore, if, because of a change in the environment, some of these isolating mechanisms are broken down, others are likely to take their place, due simply to the advantage of preventing the wastage of gametes which would otherwise result. *D. pseudoobscura* and *D. persimilis* have reached this stage of genetic divergence. In the experiments here described, ecological isolation was completely eliminated by keeping all flies in a small cage with only one type of culture medium. Existing sexual isolation was greatly weakened by keeping the cage at a low temperature. In this way, what are probably the two most important isolating mechanisms keeping the two species apart in nature were largely eliminated, yet in a surprisingly short time new isolation (in this case at least partly sexual) was built up, which brought the number of hybrids again to a low level. This change, of course, was aided by the practice of removing the hybrids entirely each generation, in this way simulating complete hybrid inviability. This, however, could hardly have had much effect, because of complete male hybrid sterility and strong backcross inviability under cage conditions. This seems to me to typify the true genetic relation of a good species to related forms. By virtue of its isolating mechanisms, in part at least maintained by selection, it is genetically independent of other organisms. In its further evolution, it is on its own, dependent entirely on its own mutations, since it is unable, as is a subspecies, ever to acquire advantageous genes from other populations.

Summary

Using artificial mixed populations of *Drosophila pseudoobscura* and *D. persimilis*, it has been possible to show, over a period of several generations, a very rapid increase in the amount of reproductive isolation between the two species as a result of natural selection. This isolation has been shown to be at least partly sexual. The implications of these findings for theories of speciation are discussed.

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THE ROLE OF THE ANTENNAE IN THE MATING BEHAVIOR OF FEMALE *DROSOPHILA*

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INTRODUCTION

In the course of studies of the ethological isolating mechanisms between different species of *Drosophila* it became apparent that either males or females or both sexes had the ability to discriminate between mates of their own and of closely related species. Further work showed that in some crosses discrimination by both sexes was involved (Spieth, 1949), but that in other crosses (e.g. *melanogaster* and *simulans*, and *D. pseudoobscura* and *persimilis*) discrimination was exercised primarily or entirely by the female (Sturtevant, 1921; Mayr, 1946; Streisinger, 1948; Bateman, 1948; Merrell, 1949). In these experiments males appeared to court their own ("homogamic") females and alien ("heterogamic") females indiscriminately, but the former proved very much more receptive. It appeared probable that females could perceive the homogamic males through specific sensory stimuli. The work here reported deals with an exploration of the nature of these stimuli.

DESCRIPTION OF EXPERIMENTS

In *Drosophila melanogaster* it has been shown (Spett, 1932; Diederich, 1941) that normal wild type females are averse to mating with yellow (y) males. On the other hand, normal males do not discriminate between normal and yellow females. Previous studies (Mayr and Dobzhansky, 1945; Wallace and Dobzhansky, 1946) have indicated that with the known exception of *D. subobscura*, visual stimuli are not essential for normal mating and species discrimination in those species of *Drosophila* that have so far been studied. Although more work on the role of vision in the mating of *Drosophila* is desirable, it was decided to concentrate in the present studies on the olfactory sense which in *Drosophila* is localized in scent organs in the third segment of the antenna (Barrows, 1907). Removal of the antennae deprives *Drosophila* of its sense of smell (Flügge, 1934). The quickest way to perform this operation is to hold the head of the etherized fly in position with a fine hair brush and to snip off (or tear off) the antennae with a fine watchmaker's forceps, such as used in embryological work. The antennae of wild type *D. melanogaster* females were removed to test the hypothesis that such antennae-less females would be less able to perceive the presumably different smell of y males. On the basis of this hypothesis it was to be expected that if experimental and control females were exposed to y males a much higher percentage of experimental than of normal females would be inseminated. The result was exactly the opposite (table 1). Although the percentage of inseminated control females was much smaller than it would have been if these same females had been exposed to wild-type males, the percentage of inseminated experimental ("antennae-less") females was even lower.

TABLE 1. *Number of inseminations of* D. melanogaster *females*

Males	Wild-type females			
	No antennae		Normal	
	+	−	+	−
Yellow	4	85	38	48
Wild type	6	38	41	7

The wild-type flies were of the Oregon R stock. 10 males and 20 females (all 3–6 days old) were dissected not less than 4 hours after the beginning of the experiment at 24.5°.

+ = inseminated, − = not inseminated.

The removal of the antennae did not seem to reduce the life span of operated flies materially. However, it appeared possible that the reduction of mating frequency in the antennae-less flies was due not to a specific role of the antennae, but rather to a general lowering of vitality as a result of the operation. To test this possibility a second set of experimental females was subjected to a much more severe operation, namely the removal of the proboscis. Since such flies are unable to drink or feed and die within 24–30 hours, they must be exposed to the males soon after awakening from etherization. In spite of all these handicaps such proboscis-less flies mate with a somewhat higher frequency than antennae-less flies (table 2). The most significant of these seven tests were those in which the flies were exposed to males soonest after the operation, before they had become very much desiccated.

The fact that the moribund proboscis-less flies mated as frequently or more so than the antennae-less flies confirms the assumption that the antennae have a specific role in the display between the sexes leading to successful copulation. Although the experimental removal of the antennae achieves the desired result of preventing the operated females from smelling the specific smell of the yellow males, it also prevents these females from perceiving the male smell itself. Not being stimulated by this male smell they become only very slowly and incompletely receptive.

TABLE 2. *Number of inseminations of* D. melanogaster *females either without proboscis or without antennae*

Males	Females			
	No proboscis		No antennae	
	+	−	+	−
Yellow	1	7	2	7
	2	8	0	10
	3	4	4	7
	6	3	3	7
Total	12	22	9	31
Wild type	4	6	0	10
	8	2	7	3
	6	7	3	9
Total	18	15	10	22
Grand total	30	37	19	53

Stocks and experimental conditions as in table 1.

The inferred reluctance of antennae-less females to mate was confirmed by direct observation of virgin flies in an observation chamber as previously described (Mayr, 1946).

Observation 1: Several five-day old wild-type males are introduced into the chamber with four antennae-less and three normal females. The males display actively to both kinds of females, perhaps even more to the operated flies which appear slightly more lethargic than the other. In spite of frequent genital contacts no copulation with antennae-less females is completed. The normal females copulate after 4 minutes, 23½ minutes, and 28 minutes. The antennae-less females had been operated two days previously.

Observation 2: As above (new virgin females), but males yellow. Males display very actively to both types of females but achieve only one copulation with a normal female during a 35 minute period. Normal females indicate some stimulation by partially spreading wings. Antennae-less females keep their wings tightly closed.

Observation 3a: Three wild-type and three yellow males with four antennae-less females. No copulation during 35 minutes despite very active displaying. There is no visible difference in the amount of courting activity of wild-type and yellow males.

Observation 3b: Four normal females added. Copulations after 4 minutes,

10 minutes, and 15 minutes, in each case between wild-type male and normal female.

Previous work (Mayr, 1946) had indicated that ethological isolation between *Drosophila pseudoobscura* and *D. persimilis* was the joint result of a number of factors, all of them combining to prevent females from being receptive to the display of alien males. If this is the case then it should be possible to show by an adequate experimental set-up that even antennae-less females mate less frequently with yellow than with wild-type males. This could be done by exposing such females simultaneously to yellow and wild-type males, and determining the frequency of either type cross either by direct observation or by genetic test. Direct observation is impractical in view of the enormously reduced sexual excitability of antennae-less females. Not a single copulation may occur in many hours of observation. The genetic test, on the other hand, is slow and tedious and leads to ambiguous results in the case of double inseminations. In view of the difficulties of these methods, an indirect approach was chosen. Two parallel sets of vials were set up, one set with yellow and one with wild-type males, in which these males were exposed for a twenty-four hour period simultaneously to normal and to antennae-less females. The results which are tabulated in table 3 show that after such a long exposure sixteen of the twenty-one antennae-less females had been inseminated by normal males, but only three of thirty by yellow males. This suggests that in spite of the loss of the antennae, the females had retained some of their ability of discriminating between wild-type and yellow males.

TABLE 3. *Number of inseminated females of* D. melanogaster

Males	Females exposed to males for 24-hour period			
	No antennae		Normal	
	+	−	+	−
Yellow	3	27	16	13
Wild type	16	5	20	2

An even better method to test the role of the antennae is to expose antennae-less females of two different species to males of one of the two. The importance of the olfactory sense can be measured through a comparison of the coefficients of isolation of normal and of antennae-less females. Exposing males of *pseudoobscura* to 409 normal females of *pseudoobscura* and to 406 females of *persimilis*, 86 per cent of the conspecific and 2.5 per cent of the alien females were inseminated. This corresponds to a coefficient of isolation (Levene, 1949) of .975. Two experimental runs were made with antennae-less females. In one run there was a deficiency of males and the food medium was partly dried out. Under such conditions even normal females of *persimilis* would show a reduced amount of insemination. In this run of nine vials 20 of 66 females of *pseudoobscura* (= 30.3 per cent) and 5

TABLE 4. *Frequency of insemination of* pseudoobscura *females and* persimilis *females by* pseudoobscura *males*

	pseudoobscura ♀		*persimilis* ♀		C. I.
	+	−	+	−	
Control (♀ with antennae)	352	57	10	396	.975
Females without antennae					
Experiment 1	20	46	5	74	.693
Experiment 2 (table 4)	34	69	30	82	.125

C. I. = Coefficient of isolation (Levene, 1949).

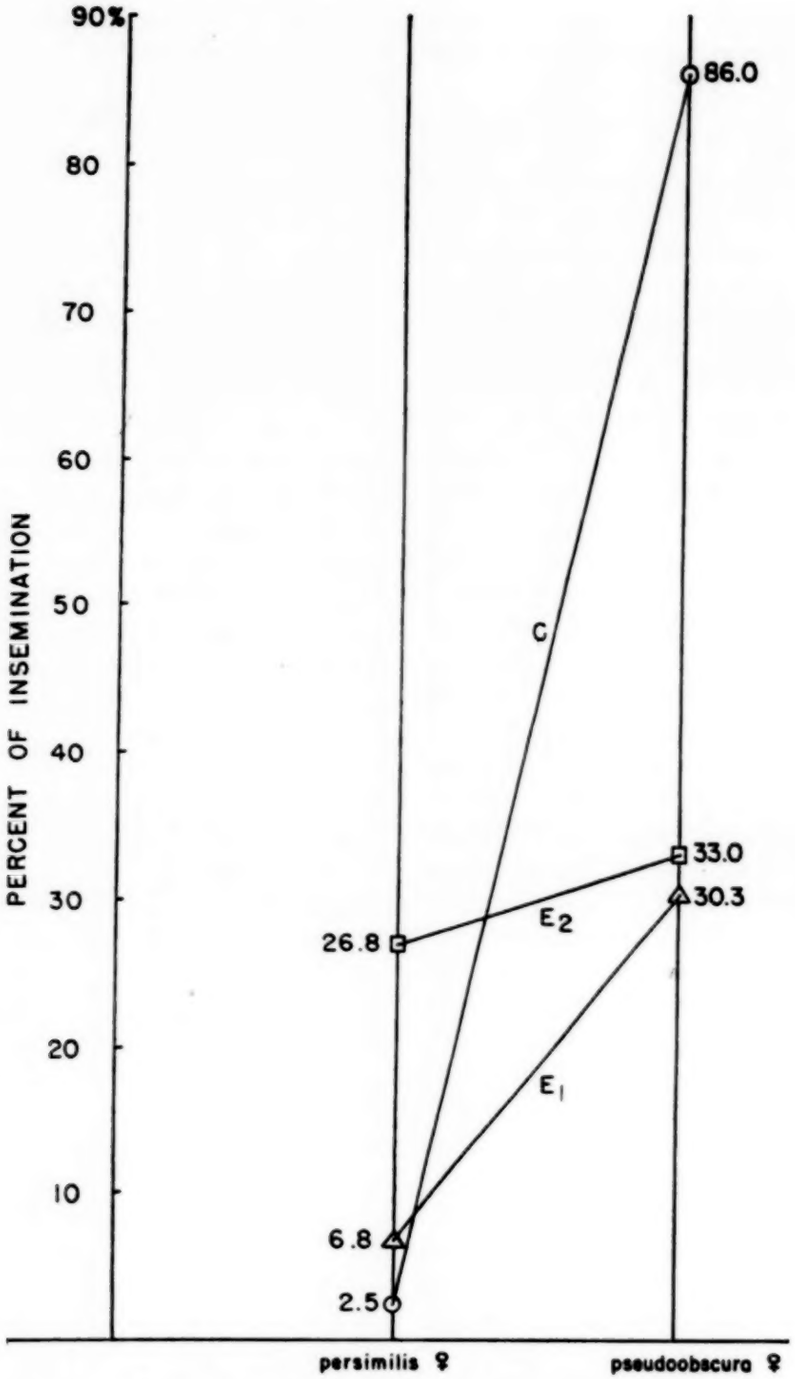

FIG. 1. Percentage of inseminated females of *Drosophila persimilis* and *pseudoobscura* exposed to males of *D. pseudoobscura*. Circles = normal females (controls); triangles = antennae-less females (experiment 1); squares = antennae-less females (experiment 2). From table 4.

of 79 females of *persimilis* (6.8 per cent) were inseminated. The coefficient of isolation is .693 (table 4). In a second set of thirteen vials run under optimum conditions on three separate days, 34 of 103 females of *pseudoobscura* (33.0 per cent) were inseminated against 30 of 112 females of *persimilis* (26.8 per cent) (table 5). The difference ($Chi^2 = 1.11$) was not significant. The coefficient of isola-

TABLE 5. *Experiment 2. Frequency of insemination by* pseudoobscura *males in all vials dissected December 15–21*

Vial	*pseudoobscura* ♀		*persimilis* ♀	
	+	−	+	−
1	2	7	2	10
2	2	6	2	6
3	3	6	4	5
4	2	4	2	4
5	4	4	1	10
6	1	6	3	6
7	2	9	0	10
8	2	3	1	4
9	5	5	3	7
10	5	4	2	6
11	2	4	3	2
12	3	4	5	4
13	1	7	2	8
Total	34	69	30	82

tion is only .125. These experiments prove conclusively that the removal of the antennae of the females greatly reduces the amount of sexual isolation between *D. pseudoobscura* and *D. persimilis*. By determining the coefficient of joint isolation it should be possible to decide whether or not the removal of the antennae eliminates all species discrimination by the females. The most significant results are obtained if an excess of males is left with the females for at least four days on fresh food medium.

DISCUSSION

The results of these observations and experiments are consistent with the hypothesis (Mayr, 1946; Spieth, 1949) that females have a certain threshold of resistance against mating which is lowered by the impact of the stimuli received from the courting male. Or, to express it reciprocally, the stimuli received from the male raise the level of receptivity of the female until it reaches the threshold of readiness for mating. The female fails to cooperate until this level is reached, and copulations attempted by the male are not completed. Very careful observations of such incomplete copulations under 10 × magnification seem to show that the male hits the female genital opening very accurately with the thrusts of his phallus but cannot penetrate because the female holds it tightly closed. This interpretation agrees with earlier observations which indicated that a slight extrusion of the genitalia by the female often preceded copulation. Such a slight relaxing of the genital opening should not be confused with the conspicuous extrusion of the female genitalia described by Spieth (1947) which serves as a rebuttal to males.

The chief component of the courtship of the male in some species of *Drosophila*, including *melanogaster* and *pseudoobscura*, is the wing flutter. Earlier work (Mayr and Dobzhansky, 1945) had indicated that this does not serve as an auditory stimulus since removal of the wings of the males increases rather than decreases the isolation index (species discrimination by the females). This may be due to a replacement of the distance (olfactory) stimulation by proximity (gustatory-tactile) stimulation. If these proximity stimuli are more species specific than the distance stimuli or if they cause less of a lowering of the threshold of the female, an increase in the isolation index will result. Further work is needed to explore this possibility. A tentative explanation of the role of the male's wings in olfactory distance stimulation is offered by Spieth (personal communication). He believes that the fluttering wings serve like a reverse propeller throwing an air stream toward the courted female. This air, containing the scent of the male, stimulates the female and increases her receptivity.* If the male belongs to a different species or to a strain with a different odor (gene *yellow*) there will be less stimulation. A reduction in the amount of olfactory stimulation is also produced if the wings of the male are removed. Likewise, there will be reduced stimulation if the receiving organ of the female is removed ("antennae-less" females). However, since the antennae are not the only organ by which the female receives stimuli

from the male, even antennae-less females will eventually copulate. In fact, in the case of *D. melanogaster* such females still seem to be capable of a fair degree of discrimination (table 3) with the help of still unexplored sense organs.

Summary

The removal of the antennae of female *Drosophila* reduces their sexual receptivity significantly.

Sexual isolation between *D. pseudoobscura* and *D. persimilis* almost completely disappears under optimal experimental conditions if the antennae of the females are removed. The antennae function in this case as an organ of species discrimination.

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SPECIATION IN FISHES OF THE GENERA *CYPRINODON* AND *EMPETRICHTHYS*, INHABITING THE DEATH VALLEY REGION [1]

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INTRODUCTION

During the Pleistocene history of the desert basins of western North America, particularly in the Great Basin, block faulting produced dozens of independent valleys separated by precipitous mountain ranges. In Pluvial [2] periods many of these valleys held lakes and large streams, but the desiccation of postglacial times disrupted the drainages between, as well as within, the once continuous basins. Isolated populations of animals and plants resulted, and the Great Basin has become a land of relicts.

The Death Valley region, lying in the southwestern extension of the Great Basin, provides an excellent example of such disconnected drainages and faunas. During late Pleistocene time, an integrated river system spread over this desert region to unite the four now isolated basins of what may be called the Death Valley system (Map 1).

Although only a limited fish fauna inhabits this area, it is rich in material for the student of evolution and is highly endemic. The living fishes comprise 3 families, 5 genera, 10 species and about 24 kinds including subspecies. One genus, *Empetrichthys*, is endemic, and nine species are confined to the system. High endemism on the species level suggests a Wisconsin or pre-Wisconsin origin of the fauna.

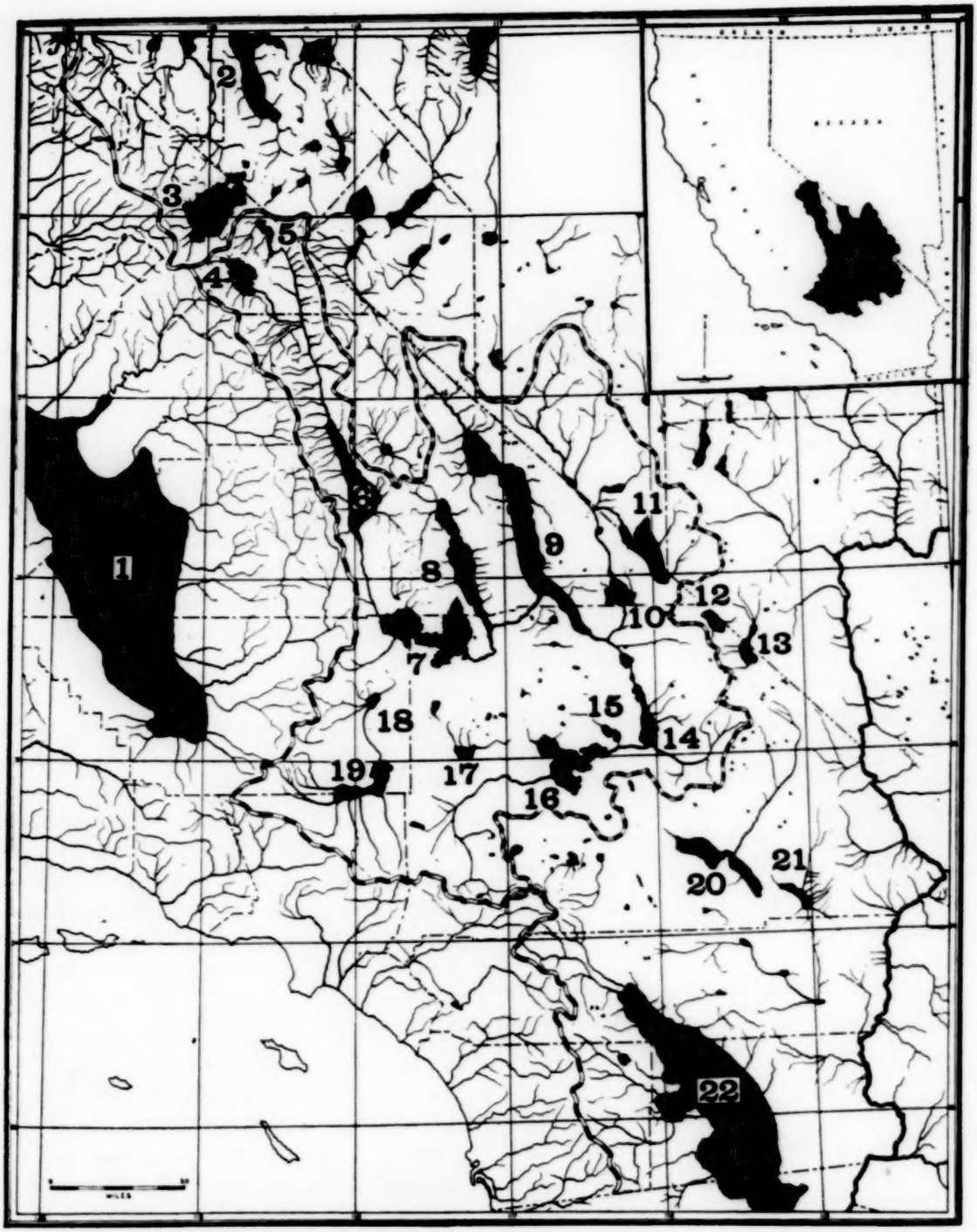
The present account is limited to a discussion of speciation in the six species represented by the cyprinodontid genera *Cyprinodon* and *Empetrichthys*, which were recently treated in detail (Miller, 1948).

DIFFERENTIATION

The genus *Cyprinodon*, which comprises small fishes that tolerate a wide variety of physical and chemical conditions, is represented by four distinct species: *C. radiosus* in Owens Valley, along the eastern base of the Sierra Nevada Range; *C. salinus* in Salt Creek, on the floor of Death Valley; *C. nevadensis* in the Amargosa River drainage system, from the south end of Death Valley to just across the California-Nevada border; and *C. diabolis* of Devil's Hole, an isolated spring in Ash Meadows, Nevada (Map 2). Each of these species is sharply differentated, and their characters run nearly the entire gamut of known variation in *Cyprinodon*, a genus found also in eastern and southern United States and southward to northern South America. All four are tied together, however, by a basic pattern of scale structure that distinguishes the species of the Death Valley system from those elsewhere (Miller, 1948, Pl. 2). This harmony in fundamental scale pattern is a striking confirmation of the interrelationship of these species, and is convincing zoological evidence to support the geological data that the waters of the Death Valley system were once continuous (Map 1). Differentiation in *C. nevadensis*, of the Amargosa River drainage, has been sufficient for the naming of 6 subspecies and the

[1] This paper is summarized from the lengthy original (Miller, 1948) with modifications and additions.

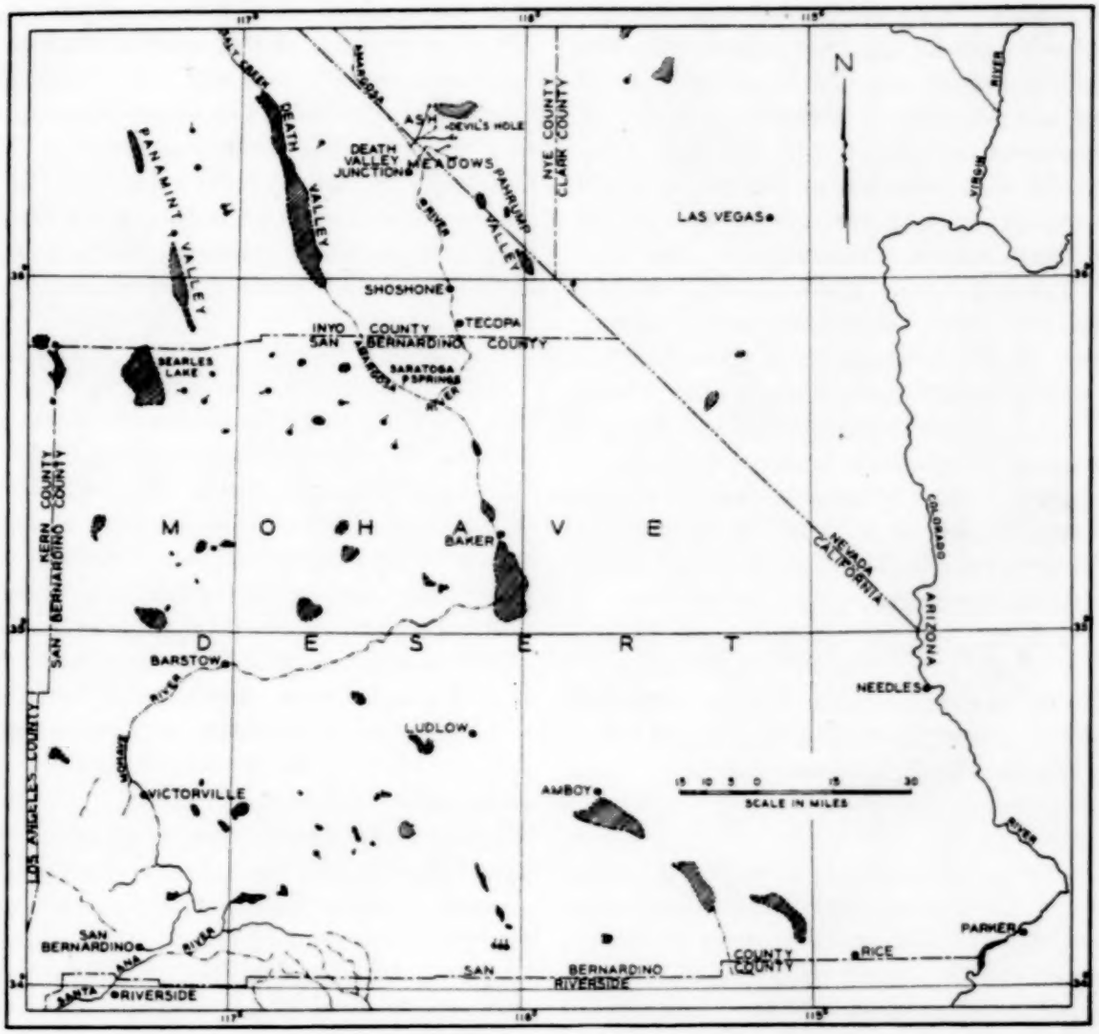
[2] The term Pluvial refers to the two or more stages, commonly regarded as of late Pleistocene (Wisconsin) age, when desert basins in western North America held lakes that have left definite records of their existence in the form of shore features.



MAP 1. Maximum extent of late Pleistocene waters in the southern Great Basin and adjoining regions. The wider boundary line marks the separation between coastal and interior or Colorado River drainages. The narrower line indicates the outline of the Death Valley system and enclosed basins (see inset). The authorities for the Pluvial lakes are cited by Miller (1946) and by Hubbs and Miller (1948).

1. Lake Tulare
2. Walker Lake arm of Lake Lahontan
3. Lake Russell [1]
4. Lake Long Valley
5. Lake Adobe
6. Lake Owens
7. Lake Searles
8. Lake Panamint
9. Lake Manly
10. Lake Tecopa
11. Lake Pahrump
12. Lake Mesquite
13. Lake Ivanpah
14. Lake Mohave
15. Little Lake Mohave
16. Lake Manix
17. Lake Harper
18. Lake Kane
19. Lake Thompson
20. Lake Amboy
21. Lake Ward
22. Lake LeConte

[1] Named by Putnam (1949)



MAP 2. Sketch map of southeastern California and adjacent areas, showing the shrunken remnants of Pluvial waters and localities mentioned in the text.

recognition of numerous races.[3] Isolated springs and a few disconnected surface flows are all that remain of this river, but during periods of heavy rain in the headwaters, Amargosa River comes to life as it pours its flood waters onto the great Death Valley salt flat—the remnant of Pluvial Lake Manly. Even during such flood stages, however, there is no evidence that the many fish populations intermingle.

The genus *Empetrichthys,* restricted to warm springs and their outflows, is represented by two species: *E. merriami,* inhabiting Ash Meadows of the Amargosa River drainage, and *E. latos,* known only from Pahrump Valley (Map 2). The latter species has differentiated into 3 subspecies. These localities, both in southern Nye County, Nevada, are isolated by a low aluvial divide. Pahrump Valley is a wholly enclosed basin, whereas during high water stages the outflows of the springs in Ash Meadows join Amargosa River, which also contains water at such times. Until recently, *Empetrichthys* was not known to have any close relative, and at one time it was thought to be related to *Orestias,* a genus peculiar to Lake Titicaca and other elevated waters of

[3] Following rather general usage in ichthyology, the term race is applied to the next systematic category below the subspecies; it does not ordinarily receive nomenclatorial recognition.

the Andes. Representatives of this remarkable genus are very rare in Ash Meadows, for only 22 specimens have been secured over a six-year period, but they are rather common in Pahrump Valley. In Ash Meadows *Empetrichthys* is associated with *Cyprinodon nevadensis* and the cyprinid, *Rhinichthys osculus*, but in Pahrump Valley it constitutes the sole native fish life. The comparative abundance of *E. latos* there is perhaps due largely to lack of competition. There may be some local differentiation in the populations of *E. merriami* inhabiting the various springs in Ash Meadows, but if so the material collected to date is insufficient to determine whether the observed variations are significant.

Factors Affecting Speciation Rates

There is evidence that at least 4 factors play an important role in modifying rates of evolution in these desert fishes.

POPULATION SIZE

In very small, closely inbreeding populations, the probability of accidental survival of particular genes or gene combinations is greatly increased. Sewall Wright has ably demonstrated the theoretical basis for this chance fixation of characters which, in the absence of interspecific competition, may be more important than selection in the evolution of such isolates. The chances for recessive mutations to become homozygous are better than they are in large populations of more widespread distribution. The determining factor is the size of the minimum effective breeding population. In some of the spring-inhibiting fish in the Death Valley region, the breeding population frequently drops to a minimum of several hundred individuals or considerably less. The most striking example is afforded by *Cyprinodon diabolis*, a dwarf species confined to a single hot-spring hole where the entire population occupies a habitat about 20 feet long, 8 feet wide, and a few inches to a few feet deep. Since every fish is in view at one time, reliable estimates of the total population are possible. The number of individuals of this species fluctuates between about 50 and 400. At times the minimum effective breeding population may thus fall below 50 individuals. No doubt the Sewall Wright effect has been a very significant factor in the evolution of this remarkable species, which is one of the most distinctive in the genus.

DURATION OF ISOLATION

The postpluvial disruption of rivers, lakes, and springs in the arid West reulted in the separation of fish populations into isolated stocks, some of which have subsequently differentiated into distinct species and subspecies. Others have attained only the racial level of differentiation. It may be stated as a general rule, for desert fishes, that a positive correlation exists between duration of isolation and extent of differentiation (Hubbs and Miller, 1948). In the Death Valley region, those populations of *Cyprinodon* estimated to have been isolated since early Wisconsin time are now distinct species, sharply differentiated from their nearest relatives. If placed in the hands of a systematist unfamiliar with the group, they might readily be regarded as comprising two or three genera. On the other hand, the close relationship among the six subspecies and the many races of *Cyprinodon nevadensis* in the basin of Amargosa River indicates recency of isolation. Geologists believe that the Amargosa may have been a continuous river as recently as a few centuries ago, at most a few thousand years ago. Nevertheless, we cannot be sure that postpluvial disruption of drainages accounts for all such speciation, since permament rivers today in other parts of the West also contain endemic forms.

The duration of time since Pahrump Valley and Ash Meadows (of the Amargosa River basin) have been isolated from each other cannot be reliably estimated in the absence of detailed studies of the past hydrography of these two regions. The isolation has been of sufficient dura-

tion to allow for the differentiation of a species of *Empetrichthys* in each basin. In Pahrump Valley, the evolution of 3 subspecies of *E. latos* in isolated springs suggests that the lake which formerly covered the valley floor was of late Pleistocene age, and the physiographical evidence supports this view. Two of the springs are less than 1 mile apart and the third is only 7 miles distant.

ECOLOGICAL CONDITIONS

Ecological conditions in desert springs are believed to play an important role in hastening evolution. These springs are very diverse, physically and chemically, and their contained fish life presumably has become highly adapted to this great variety of conditions (Sumner and Lanham, 1942). It may be assumed that the conditions in each spring and creek in some way influence the development of the remnant populations. What effect these chemical and physical characteristics may have in modifying the characters of the fishes is known only for two striking features of springs and creeks, namely, high temperature and salinity. Some of the springs in the Death Valley region are remarkably high in boron content, but no investigation has been undertaken to determine what effect, if any, this element may have on the fishes.

Much evidence has been accumulated to demonstrate that high temperature plays an important role in accelerating the rate of speciation (Plough, 1942, and other authors in same symposium). The repetition of certain striking trends in warm-spring fishes is a significant expression of this relationship: (1) the number of meristic segments is generally reduced; (2) the position of the dorsal fin is often more posterior; (3) the head, eye and other anterior parts of the body are enlarged; (4) the size is very frequently reduced; (5) the pelvic fins and rays of *Cyprinodon* tend to be reduced in size and number, or the fins may be entirely lacking; and other modifications have been observed. That some or all of these trends are genetically determined, at least in part, is indicated by preliminary breeding experiments and by the more carefully controlled work on *Fundulus* by Gabriel (1944). These show that F_1 generations retain essentially the same meristic characters found in the natural stocks. A few exceptions, however, indicate that altered environment, as well as heredity, is a factor determining the number of parts.

Observations and experiments have shown that there is a very general correlation between temperature and certain meristic characters in fishes. Thus an increase in temperature usually is associated with a decrease in number of fin rays, scales or vertebrae, and, vice versa, in cooler waters the number of segments is typically increased. This picture is oversimplified, however and citing temperature as the only, or major, causal factor is treading on uncertain ground. Many other factors undoubtedly operate in conjunction with accelerated temperature, but what these factors may be and how they affect the organism remains to be determined.

Among certain subspecies and races of *Cyprinodon nevadensis*, a striking lack of correlation between temperature and meristic characters may be demonstrated (table 1). For example, in comparing *pectoralis* with the race of *mionectes* inhabiting the source pool of Point of Rocks Spring (columns 2 and 4, table 1), it is seen that the meristic characters are consistently and significantly higher for *pectoralis* even though it inhabits the warmer of the two springs. This suggests that high temperature may have accelerated the production of mutations and that the random inheritance of these changes may have produced the variant structure of such populations. Plough (1942: 14–15) has shown that high temperature, as well as temperature shocks, greatly increases the mutation rate in *Drosophila*.

Although no clear-cut correlation between temperature and meristic characters can be demonstrated by comparing distinct subspecies or races within one

area or between two regions, a general correlation of this nature is evident. This is epecially true when populations confined to the limits of a single spring and its outlet are considered. Here one finds a significant positive correlation between temperature and certain meristic characters. This correlation is particularly well illustrated by the number of scales around the body (fig. 1). In the samples from the spring outlets, which are consistently cooler than the source springs, the body scales are significantly increased when compared with the fish from the springs. In each complex of spring source and outlet there appears to be a distinct genetic stock, the members of which respond to temperature differences in the same way. Although the spring sources may have identical or closely approximated temperatures, the average number of body scales is often not the same. For example, the temperature of Hidden Spring is nearly identical with that of Eagle or of Deep Spring, but the average number of body scales differs significantly from the samples from either of those springs (fig. 1).

Increasing salinity seems to have about the same effect on speciation as decreasing temperature, namely an increase in certain meristic parts. In *Cyprinodon salinus*, the species that inhabits the briny waters of Salt Creek on the floor of Death Valley, the scales are smaller than in any other known species of the genus. In the Death Valley race of *Cyprinodon nevadensis amargosae*, found in the waters of Amargosa River, which are less saline than those of Salt Creek, the scale counts

TABLE 1. *Correlation between certain meristic characters and temperature in five warm-spring populations of* Cyprinodon nevadensis

Means are given above, and number of specimens and standard error of the mean below in parentheses.

Subspecies Locality[1] Temperature	*shoshone* A 30° C.	*mionectes* B 31° C.	*mionectes* C 31° C.	*pectoralis* D 33° C.	*calidae* E 37° C.
Anal rays	10.07 (133, ±.04)	9.62 (103, ±.05)	9.88 (67, ±.05)	10.03 (100, ±.04)	10.08 (100, ±.04)
Pectoral rays	15.99 (214, ±.04)	15.68 (193, ±.05)	15.54 (233, ±.04)	17.10 (200, ±.04)	15.88 (200, ±.05)
Pelvic rays[2]	4.98 (258, —)	2.99 (206, —)	5.01 (234, —)	5.12 (200, —)	5.33 (200, —)
Lateral scales	25.72 (116, ±.05)	24.59 (95, ±.06)	25.40 (67, ±.07)	25.42 (90, ±.06)	25.58 (100, ±.05)
Dorsal to pelvic scales	9.39 (48, ±.08)	8.75 (36, ±.07)	9.14 (49, ±.08)	9.79 (49, ±.08)	9.35 (94, ±.06)
Dorsal to anal scales	8.88 (50, ±.06)	8.50 (50, ±.07)	8.86 (50, ±.07)	8.94 (50, ±.05)	8.94 (100, ±.05)
Predorsal scales	18.50 (111, ±.11)	17.96 (50, ±.13)	17.82 (67, ±.12)	17.91 (90, ±.12)	17.71 (99, ±.10)
Caudal peduncle scales[2]	15.64 (84, —)	13.00 (95, —)	14.34 (67, —)	15.44 (89, —)	15.71 (100, —)
Body scales	23.25 (84, ±.17)	20.44 (91, ±.12)	23.10 (67, ±.22)	24.75 (89, ±.19)	23.06 (100, ±.10)

[1] A = Outlet, Shoshone Spring
B = Source, Point of Rocks Spring
C = Big Spring
D = Lovell's Spring
E = Outlet, South Tecopa Hot Spring
A and E lie about 8 miles apart in the Middle Amargosa River basin, California; B, C, and D are 1½ to 9 miles apart in Ash Meadows, Nye County, Nevada (Map 2).

[2] The standard error of the mean was not calculated for these data as they present a markedly skewed curve and the derivation of this statistic assumes a normal frequency curve (Miller, 1948: 15).

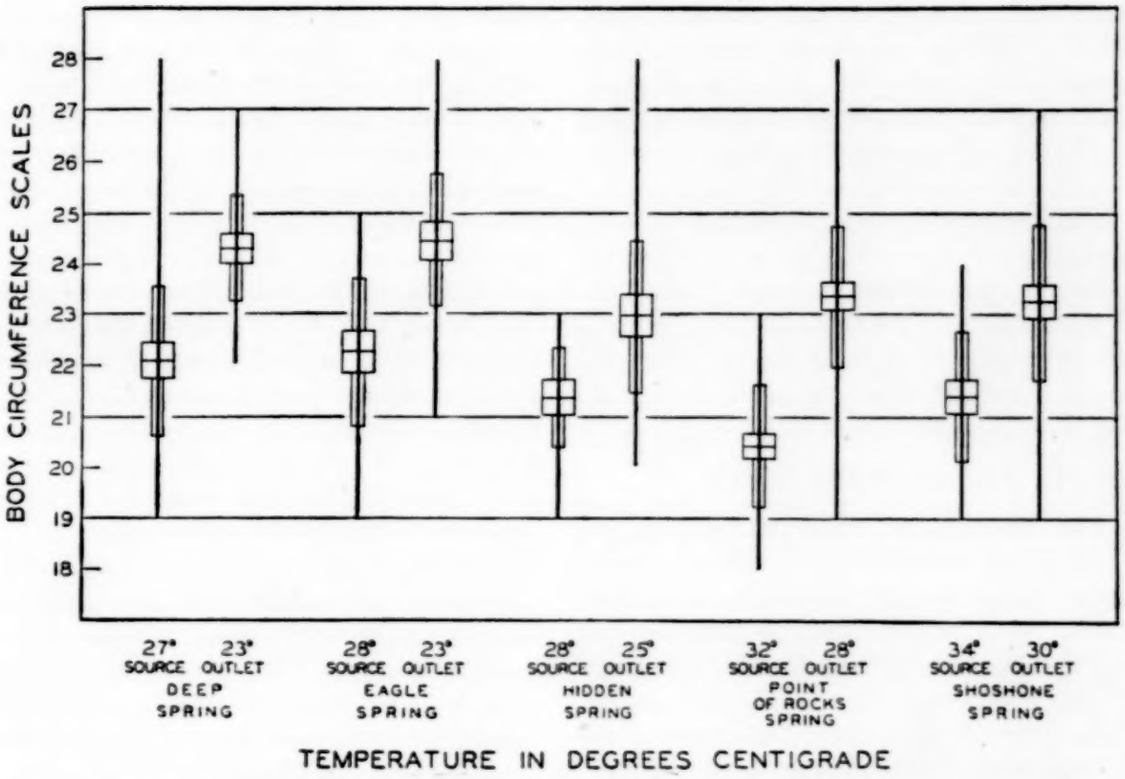


FIG. 1. Correlation between body circumference scales and temperature in certain populations of *Cyprinodon nevadensis* from the Amargosa River basin.

average higher than in any other form of *C. nevadensis*. In the spring-fed lakes at Saratoga Springs, Death Valley, where *C. nevadensis nevadensis* spawns, the waters are only moderately salty and the average number of scales around the body is lower than it is at either of the preceding localities. A direct, environmental influence is indicated, but it is thought that the observed differences are due in part to genetic adaptations that parallel those due to the direct effect of the surroundings.

NUMBER OF GENERATIONS PER YEAR

Speciation is evidently a particularly rapid and extensive process in warm springs. The role that high temperature may play in hastening this process already has been discussed. A secondary and highly significant effect of raised temperatures is the increase in number of generations that are produced each year by the warm-spring fishes.

The numerous warm springs of the Death Valley region have temperatures that vary from about 22° to 43° C. (72° to 109° F.), although fish do not tolerate temperatures higher than 40° C. (104° F.). Individually the temperature of each spring fluctuates very narrowly over the years (Miller, 1948: 130). There is evidence that *Cyprinodon* (and presumably *Empetrichthys* also) spawns every month of the year in the warmer springs (those 28° C. or higher), whereas the limits of the breeding period for the stream populations are approximately from April to October. In contrast to the 2 (or perhaps 3) generations produced per year by the stream stocks, as many as 8 or 10 generations may be the annual production for the warm-spring fish. Experimental studies have shown that *Cyprinodon nevadensis* may reach maturity and produce young when only 2 months old. Since these fish were born and developed in much cooler water than that of their native warm springs, it seems safe to as-

sume that the warm-spring fish are capable of breeding in nature when only 1 month or 6 weeks old. This production of numerous generations each year may greatly accelerate the time rate of evolution of such populations.

The isolation of small, breeding populations that are subjected to an accelerated mutation rate and an increase in number of generations, due to high temperature, are believed to result in the striking differentiation exhibited by such species as *Cyprinodon diabolis*.

Experimental Work

The rearing of three subspecies of *Cyprinodon nevadensis* in concrete pools, under physical and chemical conditions unlike those in their natural habitats, has led to some interesting results that need further checking. With only few exceptions, analysis of meristic characters of the F_1 has demonstrated that these generations retain the characters of their respective subspecies in nature. In general, the average values for the first generations of the three subspecies lie in the same direction as do the values for the natural stocks of these subspecies. If the subspecies in nature do not differ significantly in a particular count, the values for the F_1 stock likewise are not significantly different.

Interspecific, as well as intraspecific, hybridization was carried out. When species of *Cyprinodon* were crossed, lowered fertility generally resulted, particularly in certain reciprocal crosses. Attempts to mate males of *Cyprinodon salinus* with females of *Cyprinodon macularius* (a species confined to the lower Colorado River basin) failed. Mating females of *salinus* with males of *nevadensis* resulted in several generations of hybrids, but comparatively few fish were produced. The most successful interspecific matings took place between *nevadensis* and *macularius*, but the success of some of these combinatinons varied with the reciprocal crossings. For example, when males of *C. macularius* were crossed with females of *C. nevadensis amargosae*, only moderate success was obtained, but when males of *amargosae* were hybridized with females of *macularius*, very good results ensued. Some of the variations in productivity were probably influenced by differences such as size, temperature, and exposure, in the various pools used in the experiments.

In the hybridization between subspecies of *C. nevadensis*, best results were obtained when the two spring forms (*nevadensis* and *shoshone*) were *crossed*. The two other combinations, *amargosae* × *nevadensis* and *amargosae* × *shoshone*, involved one parent form (*amargosae*) that develops in nature in saline water. The experimental pools contained fresh water. Whether this factor had an important bearing on the poor results when *amargosae* was used as one parent, or whether *nevadensis* and *shoshone* are more closely related to each other than either one is to *amargosae*, is problematical.

Paleontological Evidence

Although the paleontological data on fishes from western United States are woefully incomplete, a study of the few available Miocene fossils reveals a fauna quite unlike that now inhabiting the region. It is therefore assumed that the recent fish fauna is not much older than Pliocene. Pleistocene fish remains should be of great importance in working out the more recent history of the fish fauna, but the few specimens collected thus far have been largely fragmentary.

The occurrence of 3 species of *Fundulus* in Death Valley and the Mohave Desert in late Pliocene or early Pleistocene time, and of a fossil *Cyprinodon* in Death Valley during the same period (Miller, 1945), is of significance in attempting to elucidate the origin of the cyprinodontid fauna. *Fundulus* and *Cyprinodon* are widespread today in regions of low altitude and are particularly abundant near and along coastal areas. In particular, the presence of *Fundulus* suggests a lowland connection to the sea from the southwest, as the only living members of this genus in the

Pacific drainage are now found from Monterey Bay, California, nearly to the tip of Lower California. *Cyprinodon* clearly came in from the southeast, in the direction of the Colorado River (Miller, 1946).

The endemic genus *Empetrichthys,* whose nearest living relative is *Crenichthys* of eastern Nevada, is evidently an old (pre-Pleistocene?) relict. Both genera were presumably derived from *Fundulus,* and *Empetrichthys* may have descended from a species similar to *F. curryi,* one of the fossils from Death Valley (Miller, 1945: fig. 1).

Summary

Speciation in the cyprinodontid fishes inhabiting the Death Valley region is correlated with the disruption of waters since late Pleistocene times, with population size, with the number of generations produced each year, and with physical and chemical conditions of the remnant springs and creeks. There are two living genera, *Cyprinodon* with 4 species, one of which has 6 subspecies, and *Empetrichthys* with 2 species of which one has 3 subspecies. All of the species and subspecies are endemic, as is *Empetrichthys* which is evidently an ancient relict. Three fossil representatives of *Fundulus,* probably of late Pliocene or early Pleistocene age, indicate a lowland connection to the southwest. One of the species may have been near the ancestral stock from which *Empetrichthys* was derived. The *Cyprinodon* reached Death Valley from the southeast, by way of the Colorado River basin or its antecedent. Most of the subspecies of *Cyprinodon nevadensis* show racial differentiation, particularly *C. n. mionectes* of Ash Meadows, Nevada. High temperature is believed to have accelerated the time rate of evolution in warm-spring populations by increasing the mutation rate and the number of generations produced each year. Many of the local subspecies and races have very small ranges, which may be closely approximated. One unique species, *Cyprinodon diabolis,* is confined to a single small spring in which its total numbers fluctuate from about 50 to 400 individuals. Experimental studies have demonstrated that F_1 generations of pure stocks of three subspecies of *Cyprinodon nevadensis,* reared under environmental conditions greatly altered from those of their natural habitat, maintained essentially the same meristic characters as are found in the natural populations. A few exceptions, however, indicate that altered environment, as well as heredity, is a factor in determining the number of segments.

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PARALLEL AND DIVERGING SKELETAL EVOLUTION IN VERTEBRATES AND ARTHROPODS

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INTRODUCTION

Sixty-five years ago, in 1884, William Patten of Dartmouth College was already engaged in his investigations on the comparative morphology of arachnids and vertebrates; this culminated in his monumental work of 1912, "The Evolution of the Vertebrates and Their Kin."

After frequent study of this book ever since its publication, I regard it with profound respect even though I am forced to dispute its outstanding thesis, which was that the vertebrates had been derived from early Palaeozoic marine arachnids, the existing *Limulus* being a rather primitive survivor.

REVERSED DORSO-VENTRAL ASPECTS OF THE BODY IN ARTHROPODS AND VERTEBRATES

Patten's predecessors, from the time of Étienne Geoffroy St. Hilaire onward, saw that the ventral side of an insect corresponded functionally with the dorsal aspect of a vertebrate. All those who wished to derive vertebrates either from annelids or from arthropods did not hesitate to accept this as a prime condition. Patten also adopted it, and in his comparative diagrams all the arthropod stages were shown as if they were swimming on their backs, that is with their paired appendages pointing upward.

SIMILAR GROWTH FACTORS IN DIFFERENT PHYLA

Adult vertebrates agree with *Limulus* and other arthropods in having dorsaventrally differentiated and bilaterally symmetrical bodies, as well as in the absence of the features listed in table 1, columns 1, 2, 3, 6, 7. Thus the body forms of vertebrates and marine arachnids share five or more sets of similar limiting growth factors, controlling many of their respective adaptations to gravitation, to the surrounding medium or to the substratum. The researches of D'Arcy Thompson (1942) and of Breder (1947) suggest that similar potential growth factors in the hereditary patterns would produce corresponding similarities in body form and general appearance, even in members of widely different stocks. For example, it has long been recognized that the parts of the complex heads of cephalopods are not at all homologous with those of vertebrates.

EXTERNALLY JOINED METAMERIC APPENDAGES

Partly for this general reason I feel that Patten was begging the question when he assumed that the cephalo-thoracic segments of embryo arthropods, with their jointed paired appendages, were collectively homologous with the branchial arches, external gills and cephalic neuromeres of amphibian larvae; because the paired appendages of arthropods are externally articulated, exoskeletal structures developing on the neural aspect of arthropod embryos, whereas the branchial arches of primitive vertebrates develop beneath the brain and are in line with the heart, on the haemal side facing the yolk.

Patten regarded the jointed externally armored pectoral appendages of the Devonian vertebrate *Bothriolepis* as comparable with the "balancers" of *Amblystoma* and other urodeles. In his figure 302 (p. 466), the "transitional stage E" based partly on *Amblystoma*, the balancers are represented as comparable with the long oar-like cephalic appendages of the "generalized arachnid" or merostome

TABLE 1
ADULT BODYFORM AND SKELETON

	Global (g) cupshaped (c) radiate (r)	Body ciliate (ci) stalked (s) tentacles (te)	Five rayed	Bilateral symmetry (bs) tapering to tail (tap)	Dorso-ventral differentiation (dv)	Helicoid to spiral	Pseudopodia	Parapodia	Externally articulated or ringed (r) legs in pairs	Myomeres en chevron	Exoskeleton or crust, stiff (stf) pliable (pli) or intermediate (int)	Bone cells present	Notochord (ntc) or vertebrae (vert)	Pectoral (pct) or pelvic (plv) limbs	
PROTOZOA															
PHIZOPODA	gcr	0	0	few	some	some	×	0	0	0	some	0	0	0	
MASTIGOPHORA	gcrs	cl.fl.	0	few	some	some	×	0	0	0	pli	0	0	0	
COELENTERATA															
HYDROZOA	gcrs	tcl	0	0	×	0	0	0	0	0	var	0	0	0	
SCYPHOZOA	gcr	tcl	0	0		0	0	0	0	0	pli	0	0	0	
ANNULATA															
POLYCHAETA	0	tcl	0	×	×	one	0	×	0	0	pli	0	0	0	
MOLLUSCA	0	tcl	0	some	×	×	0	0	0	0	stf pli	0	0	0	
ONYCHOPHORA	0	0	0	×	×	0	0	×	r 14-42	0	× pli	0	0	0	
TRILOBITA	0	0	0	×	×	0	0	0	× 2-44	0	×	0	0	0	
ARACHNIDA															
LIMULUS	0	tcl	0	×	×	0	0	0	4 × 11	0	× int	0	0	0	
INSECTA	0	0	0	×	×	0	0	0	× 3	0	× var	0	0	0	
BRACHIOPODA	0	0	0	×	×	0	0	0	0	0	×	0	0	0	
ECHINODERMATA	r	tcl	×	× some	×	0	0	0	0	0	× stf pli	0	0	0	
TUNICATA															
ASCIDIA	bag	0	0	part	×	0	0	0	0	0	× var	0	ntc larv	0	
HEMICHORDA	c some	tcl some	0	bs tap some	×	0	0	0	0	0	× pli	0	?ntc	0	
CEPHALOCHORDA															
AMPHIOXUS	0	0	0	×	×	0	0	0	0	×	pli	0	ntc	0	
OSTRACODERMI	0	0	0	×	×	0	0	0	0	×	× var	× some	ntc	pct some	
ELASMOBRANCHII	0	0	0	×	×	0	0	0	0	×	× var	0	ntc vert	pct plv	
OSTEICHTHYES	0	0	0	×	×	0	0	0	0	×	× var	×	ntc vert	pct plv	
PRIMITIVE TETRAPODA	0	0	0	×	×	0	0	0	0	×	× var	×	ntc vert	pct plv	
REPTILIA	0	0	0	×	×	0	0	0	0	×	× var	×	ntc vert	pct plv	
MAMMALIA	0	0	0	×	×	0	0	0	0	×	× hairs	×	ntc vert	pct plv	Bradley del 1925

(D). But, the amphibian balancers, according to Patten's own figures 168, 169, are outgrowths of the outer lip-jaw (maxilla) in front of the branchial arches while the paired appendages of *Bothriolepis* and other pterichthyids articulated with the antero-ventro-lateral plate of the shoulder girdle, behind the head and behind the branchial arches.

In *Bothriolepis*, the large externally jointed pectoral fins are far more probably homologous with the pectoral lobes or fins

of the cephalaspid ostracoderms, as clearly shown by Stensiö (1945b), and their assumed homology with the large swimming appendages of cladoceran crustaceans is now clearly unwarranted.

On the flat ventral surface of the cephalo-thoracic shield of *Trematapis schmidti,* which Patten himself had collected in the Upper Silurian of the Island of Oesel in the Baltic Sea, he found a series of "incisures on either side, diminishing posteriorly and curving inward." Associated with these remains were certain very small plates (fig. 241, figs. C, D, E, F) which he regarded (1912, p. 362) as ". . . portions of the armored appendages. . ."; these, he thought, fitted into the incisures. On one of his later collecting trips to Oesel however he discovered specimens of *Trematapis* in which these two small plates were practically in place, the one proving to be a dorsal fin scute, the other a ventrally situated or anal scute (Patten, 1931). This determination which was accepted after careful study by Robertson (1938, p. 178) disposed of the idea that these pieces were parts of cephalic appendages.

The ventral incisures in *Trematapis* clearly suggest the curved series of small circular gill-openings in the anaspid ostracoderms, which, in turn, compare well with the known gill-pores of the living cyclostome *Petromyzon.* Accordingly there seems to be no known sound palaeontologic evidence of externally-jointed cephalic appendages in *Trematapis.*

The presence of myomeres is highly characteristic of *Amphioxus,* ostracoderms, fishes and higher vertebrates; but these structures are only vaguely comparable with the mesoblastic somites of *Limulus* (cf. Patten, p. 229, figs. 129, 130). The branchial arches of embryonic vertebrates are indeed supplied by nerves and moved by muscles that extend downward from the locomotor somites above them, and may thus be considered to have a metameric origin; but this is far from proving that they have been derived from paired cephalic leg-jaws of the arthropod type.

The large gill pouches in cephalaspids were, as Stensiö has shown, partly separated dorsally by transverse skeletal ridges or partial plates; these may well be represented in the existing cyclostome *Petromyzon* by the dorsal bars of the branchial basket. But here, apart from their repetitiveness, there is no suggestion whatever of externally articulated locomotor appendages in this apparatus.

In the Arthropoda the paired appendages were the principal locomotor organs, both for crawling along the bottom and for free swimming. But in *Amphioxus,* the cephalaspids and the anaspids the principal organs of locomotion were the myomeres; the paired pectoral and pelvic finfolds, even although supported by dermal spines were not externally jointed appendages. Patten therefore made three assumptions: (1) that the paired, jointed appendages of arthropod type underwent a profound transformation in structure and function; (2) that those in the cephalothoracic region gave rise to the vertebrate jaws, hyoid arch and branchial arches, gill balancers and external gills; (3) that the abdominal appendages and their book-gills became vestigial (*ibid,* p. 37, fig. 34). But no trace whatever of these abdominal book-gills is known in vertebrates and as noted above the supposed homologies of arthropod cephalic appendages with vertebrate gill-arches rest on no good evidence.

Cephalization by Coalescence of Neuromeres

Patten graphically transformed a hypothetical arthropod embryonic brain into a vertebrate brain by two main processes: first, as in the young scorpion the neuromeres became enlarged and coalesced and grew downward in front (*ibid.,* fig. 47) to form the swollen forebrain. Secondly, in *Limulus,* with the great expansion of the sensitive head-shield, the brain became much larger, more complex, with folded cortex, and at the same time it

swung forward into a horizontal position in line with the following neuromeres. Meanwhile, the head-shield surrounding the front end grew upward and backward on to the neural side, gradually covering the paired cephalic appendages. Thus, he supposed, the paired eyes and pineal eyes were also carried around on to the neural, or now functionally dorsal, side of the shield. In this manner Patten ingeniously accounted for the fact that the head-shield of *Limulus* lies mainly on the haemal surface, while that of *Cephalaspis* was on the neural side. But the intermediate stages (B, C), so clearly conceived and drawn by him, remain wholly hypothetical. The same is true of his beautifully drawn figures (especially figs. 29, 33) showing how the paired appendages, which are so conspicuous on the neural side of the embryo arthropods, were gradually drawn downward and forward to form the jaws and gill arches of the adult vertebrate. But just as his proposed homologies between paired arthropod appendages and vertebrate branchial arches may be challenged as illusory similarities and arbitrary extensions of morphologic terms from arthropod to vertebrate, so too his attempt to equate the neuromeres of the nervous systems of arthropods and vertebrates rested on similar unverified assumptions.

It is gladly admitted however that his analysis of the functions of the various sense organs and their nerves in *Limulus* resulted from many carefully planned and well executed experiments on the living animals by himself and his students.

In classifying the "cranial nerves" and the parts of the brain Patten applied the

TABLE 2

LOCOMOTOR SYSTEM	LOCOMOTOR METHOD	METAMERISM	BODY-WALL MUSCLES	EXOSKELETON	ENDOSKELETON	THORACIC SHIELD
COELENTERATA OBELIA	Pulsation	0	0	0 Thin ectoderm	0	0
ANNULATA NEREIS	Lateral undulation	X	Dors. ventr., long. Oblique Circular	Cuticular	0	0
ONYCHOPHORA PERIPATUS	Mainly by paired app.	0 3 longit. comp. rim?	Longitudinal Diagonal Circular	"	0	0
MYRIOPODA SCOLOPENDRA	"	X	Much as in Insects	Chitinous	0	0
INSECTA PERIPLANETA	Right and left on alternate tripods	X	Longit. sternal Oblique " Longit. tergal	"	0	Pro-, meso-, & meta-thorax
CRUSTACEA ASTACUS	1. By sharp flexure of abdom. segments. 2. By paired app.	X	Interlooping flexor & extensor muscles	Chitinous impregnated with lime	Endophragmal plates (= inturned exoskeleton)	Fused cephalo-thorax
ARACHNIDA LIMULUS	By paired app.	X	Much as in Crustacea	"	"Endosternite" "Endocranium"	Cephalo-thorax with chitin. trabeculae; some with candliculi
PROTOCHORDATA BALANOGLOSSUS ASCIDIA AMPHIOXUS	Partly by cilia " " muscles Undulation of notochord	3 coelomic segments X X	Longit. mesoderm Mesod.ic strands Myomeres	Thin epidermis Cellulose test Thin epidermis	Small notochord Large larval " " adult "	0
OSTRACODERMI ANASPIDA CEPHALASPIDA	Lateral undulation " " + pect. fins	X X	" "	Dermal scales With bone cells	Notochord "	? Reduced Cephalothor. shield + scapulocoracoid
PLACODERMI BOTHRIOLEPIS ACANTHODII	Undulation + pect. fins " + fin folds	X X	" "	" " " Bone like strata	" -	X Carapace + plastron Reduced to dermal pectoral girdle
ELASMOBRANCHII CLADOSELACHE	" + fin folds	X	"	No bone cells, thin shagreen	Calcified cart.	0 scapulocoracoid
OSTEICHTHYES POLYPTERUS	" "	X	"	Bone cells in complex scales	Ossified "	X + scap. cor.
CHOANATA EUSTHENOPTERON	" "	X	"	"	Moderate	X + scap. cor.
TETRAPODA ERYOPS	" + pect. & pelv. limbs	X	"	Reduced	Well developed	Reduced + scap. cor. large

TABLE 3 HEAD	NERVE CORDS	BRAIN TYPE	BUCCO-PHARYNX	RESPIRATORY PARTS	JAW TYPE	CRANIUM TYPE
COELENTERATA OBELIA	Circular	0	= Oral tube	Body surface	0	0
ANNULATA NEREIS	Ventral chain, periesoph. comm.	Paired ganglia for eyes tentacles, &c.	Pharynx protrusile, with chitinous jaws	Lobes of parapodia	Chitinous infernal jaws	Prostomium + peristomium
ONYCHOPHORA PERIPATUS	Right & left lat. nerve cords	"	"	Tracheal tubes	"	Prostomium + peristomium fused
MYRIOPODA SCOLOPENDRA	Ventral chain with ganglia	Large cerebral ganglia	Pharynx suctorial, muscular	"	3 pairs app. (mand., maxl., & max. 2)	Prostomium + 7 somites fused
INSECTA PERIPLANETA	" 3 thor. ganglia enlarged	Supr. & subeso-phag. ganglia	Bucco-pharyngeal tube	"	"	Complex of 6 segments
CRUSTACEA ASTACUS	6 thoracic ganglia enlarged	"	Straining "stomach" or gastric mill (from stomodeum)	Gills from dorsal branch of thoracic app.	"	Cephalothorax including 8 thoracic segments
ARACHNIDA LIMULUS	5 pairs large pedal ganglia	Cerebral ganglia very large	Pharynx suctorial muscular	Book gills on 5 abd. app.	1 pair of chelicerae	Large cephalic shield "Cranium" = entosternite
PROTOCHORDATA CEPHALODISCUS	Small dorsal ganglia		Pharynx large, one gill slit	Plumed tentacles	0	0
BALANOGLOSSUS	Dorsal & ventral nerve strands connected by nerve ring		Pharynx large, many gill slits, supported by rods		0	"Proboscis" = prostomium or rostrum
ASCIDIA	Short dorsal nerve, &c.	Small dorsal ganglia with twigs	Huge grid of transverse and vertical tubes & vertical gill slits		0	0
AMPHIOXUS	Longit., dors., spinal nerves	Nerve tube Brain very small 2 cranial nerves	Pharynx with about 100 gill slits Looped branchial bars		0	0
OSTRACODERMI PTERASPIS	Dorsal nerve cord	Pineal and paired eyes	Oralobranchial chamber Paired gill pouches opening into operculat cavity		Small oral jaw plates	Cephalothorax with 9 plates
CEPHALASPIS	"	Essentially as in Petromyzon V1 sep. from V2	Oralobranchial chamber. Ten pairs of gill pouches (I=oral, II=hyoid) with interbranchial septa		0	Cephalothorax with bone-cells. Brain trough
CYCLOSTOMI PETROMYZON	"	Large olf. lobe " gang. hab. " infundib.	Circular lip-funnel with horny teeth Branchial basket exterior to gill pouches		Large rasp on muscular "tongue"	Olf & otic capsules; basal plate with lateral walls
PLACODERMI ACANTHODES	"	Large paired eyes Small olfactory parts	Very large oralobranchial chamber. Jaws & gill arches folding vertically. Large opercular chamber.		Large pal. qu. + Mck. + sur-face scales.	Rostrum very short. Wide across large eyes. Occiput narrow
OSTEICHTHYES SALMO	"	Small olf. lobes. Very large mid. br. " " cblm.	" " " Branchial arches also used in deglutition		" " + derm-bones	Fairly primitive bony-fish cranium
TETRAPODA FROG	"	Primitive cerebr. Optic lobes. Very small cblm.	" " " Gill clefts closed Hyoid arch = stapes + larynx	Lungs in adults Larvae ext. gills	"	Sphenethmoid. Sphen-optic + otic-occipital divisions united.

standard nomenclature of the vertebrate nervous system to what appeared to be functionally equivalent parts in *Limulus* and other arthropods. His comparative drawings convey an impression of identical plan in arthropod and vertebrate brains with only minor differences in details. But here again the real question at issue is whether such correspondences stand for true homologies between related animals or for parallel and convergent resemblances between unrelated animals having somewhat similar habits.

But since the arrangement and sequence of the parts in both brain types is conditioned by the similar sequence in functional groups of corresponding sensory, locomotor and viscersal organs in forwardly moving bilaterally symmetrical predatory animals, the resemblance between the brains of *Limulus* and vertebrate may mean no more, or no less, than the adaptive resemblances in body form and in the organs themselves. Nor do the oldest known vertebrate brain-casts, those of the cephalaspids as figured by Stensiö actually bridge the gap between the marine arachnids and the ostracoderms.

In connection with the progressive coalescence of neuromeres, which is one of the main features of the process of cephalogenesis, it may be noted that both *Limulus* and the Cephalaspids were presumably mud-living or bottom-living forms. In the case of *Limulus* its wide cephalothoracic shield may have been useful in several ways: e.g. as a stream-lined buffer against moving water and as a temporary corral for enclosed small prey,

such as marine worms, small bivalves and fragments of dead fish. In *Limulus* all this required keen sense organs, diversified appendages and an elaborate control system, for seizing, ingesting, comminuting and swallowing.

In some ways *Limulus* resembles skates and rays and it is perhaps not surprising that the haemal sensory nerves which radiate out to both neural and haemal surfaces of the thoracic shield (Patten, fig. 66 and p. 97) superficially suggest the radiating sensory nerves of skates and the so-called "electric nerves" of Cephalaspids. So too the box-like "endocranium" of *Limulus* superficially suggests those of sharks and rays. As Patten showed, the functional cranium of *Limulus* (fig. 215, p. 317) is homologous with the endosternite of the scorpion (fig. 216), but the somewhat similar brain cases of the skates, of embryo cyclostomes or of Silurian cephalaspids retain no surely identifiable traces of such derivation.

Patten's Disposal of the Protochordates

As Patten believed that the amphibian embryo possessed genuine relics of the cephalic appendages of marine arachnids, he put the amphibian-dipnoan stem in the middle of his vertebrate tree, as indicated in his figure 309.

The protochordates, in which the head is almost or quite lacking, were classed by Patten as Acraniata, in contrast with the Syncephalata, which included Myriopoda and insects on the left, the Arachnida (s.l.) and the vertebrate stem (ostracoderms) in the center, and the Trilobita and higher Crustacea (Malacostraca) on the right. These amazingly diversified branches were thus squeezed together in his diagram because of the coalescence of their neuromeres to produce a complex brain. But, if the foregoing analysis of Patten's postulates and assumptions be well founded, it would follow that Pat-

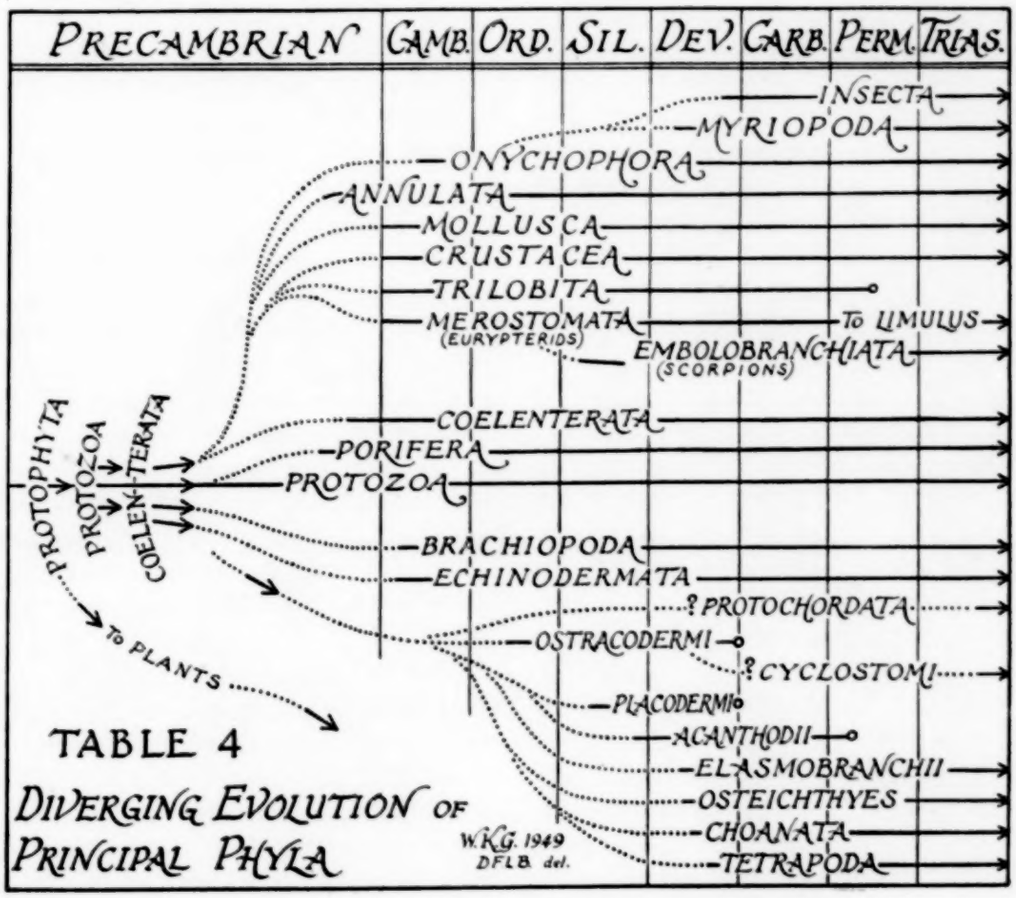

TABLE 4

DIVERGING EVOLUTION OF PRINCIPAL PHYLA

ten's Syncephalata is an unnatural group, based on parallel and convergent resemblances between members of phyla that were already diversified at the beginnings of their respective fossil records.

The foregoing considerations tend to bring the Protochordates back into the problem of the origin of the vertebrates. *Amphioxus* may well be specialized in such features as the reduction of its exoskeleton to a thin epidermis, the multiplication of its gill slits, the forward growth of the notochord to the tip of the rostrum and the excessively small size of the brain; but in the perfection of its notochord it appears to retain and even emphasize a primitive chordate feature. Patten made a sustained effort (pp. 323–336) to equate respectively the lumen of the middle cord of insects and arachnids with the central canal of the vertebrate embryonic neuron and the lematochord with the notochord; but here again he assumes an homology where in the light of other evidence only an analogy can be safely claimed. I find it difficult indeed to reconcile Patten's conception of the origin of the spinal cord and notochord of vertebrates with Conklin's very clear factual account (1932) of the development of these parts in *Amphioxus,* where a first rudiment of the notochord, arising from the center of the mesoderm crescent of the blastopore, grows rapidly forward, in the center of an involuting plug, the dorsal layers of which give rise to the spinal cord.

In short, in the light of many other well known studies of the place of the protochordates in the history of the vertebrates it seems as if *Amphioxus,* although unknown as a fossil, and specialized in habitus, is basically a primitive survivor of some very early chordate group, perhaps related to the ostracoderms (Gregory, in press).

Since Patten's time the dogma of recapitulation has been pretty well deflated by T. H. Morgan and others. But with reference especially to the lower invertebrates (e.g. Coelenterata, Annulata, Echinodermata, Brachiopda and others) it is still probable that larval or young stages may often but not always retain some earlier phylogenetic features which are lost in the adults. Thus in the well known case of the Ascidia, the larval free-swimming stage is still regarded as a more ancient type than the degenerate adults, and the same holds for the famous parasitic crustacean *Sacculina.*

Despite the diversity of most of the known phyla of animals in Lower Cambrian times (Walcott) the varied trochozoan larvae of today may well preserve the ground plan of intermediate adult stages, between the Coelenterata and the Annulata, the Onychophora and the Arthropoda. On the other side of the phylogenetic bush even such a specialized looking hemichordate as *Cephalodiscus* may well retain much of the ground plan of the several Tornaria-like Pre-Cambrian stocks which gave rise respectively to the Molluscoidea, the Echinodermata, the Protochordata and the Chordata.

Conclusions

(1) The Lower Cambrian invertebrates of numerous phyla described by Walcott included diversified annulates, crustacea, trilobites, etc., together with direct forerunners of the modern *Limulus;* but despite the partial resemblance between the Silurian *Bunodes,* representing the Limulus stock, and its contemporary, the ostracoderm *Cephalaspis,* the concensus of palaeontologists is that this resemblance does not indicate the derivation of chordates from marine arachnids.

(2) The morphologic and phyletic gap between any of the Arthropoda and the Chordata still appears to be enormous, in spite of Patten's long sustained and logically consistent effort to prove the contrary.

(3) Patten's superphyletic group Syncephalata including the arachnids and the vertebrates was an unnatural one.

(4) The protochordates are not, as Patten held, more widely removed from the vertebrates than the arachnids are, but may rather be fragments of related stocks

connecting the vertebrates with the remote roots of the Echinodermata and Brachiopoda.

(5) *Amphioxus* appears to be a specialized derivative of the ostracoderm stem.

(6) The following hypothesis is advanced: that the structural resemblances between *Limulus* and *Cephalaspis* are partly due to: (a) independent derivation from different earlier phyla which both had motile larvae bearing ciliated bands, useful for locomotion and for drawing in water containing oxygen and food particles; (b) independent progression toward pursuit of larger, active animal food, requiring organs for seizing, pulling in and breaking up the diverse prey, with correlated coalescence of nuromeres and building up of more complex brains; (c) independent evolution of a large rounded head-shield useful for protection and for pushing into sandy or muddy bottoms in shallow water.

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AUTOSOMAL LETHALS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Since gene mutations furnish the raw materials of evolution, it would seem that man's technological achievements, such as his atomic and electronic devices, may influence his biological future. Among the variables which are important in this connection are similarity of induced to spontaneous gene mutations, the degree of dominance of induced mutations, the number of gene loci which produce mutations, and the rates of mutation at different loci. Stern and Novitski (1948) have drawn attention to the semi-dominance exhibited by both induced and spontaneous "recessive," sex-linked, lethal mutant genes of recent origin in *Drosophila melanogaster*. Our data indicate that a large proportion of induced lethals in the second chromosome of *D. melanogaster* are also incompletely recessive. These data, although of a preliminary nature, are presented below.

Three experimental populations of *D. melanogaster* are being analyzed for lethal second chromosomes. The original parents of these populations were mixtures of 16 stocks of flies each of which was homozygous for a lethal-free second chromosome derived from a single Ore-R male. The original flies of two of these populations were treated with X-rays; males of population No. 1 were exposed to approximately 7,000r, females of population No. 1 and males and females of population No. 2 to approximately 1,000r. Population No. 3 is the untreated control. These populations are maintained in cages similar to the type described by Wright and Dobzhansky (1946). (A detailed description of the treatment and of the population cages will be given in a later paper.) The populations and all the cultures made during the course of the genetic analyses are kept at 25° ± 1° C.

The proportions of lethal-containing second chromosomes are determined in egg samples taken from the populations each generation (2 weeks under our experimental conditions) or, later, every other generation. A number of males which hatch from these eggs are mated individually to CyL/Pm females. A single CyL/+ male from each F_1 culture is mated once more with CyL/Pm females and several CyL/+ males and virgin females (F_2) from each of these cultures are mated to obtain F_3 flies. Since all F_2 males and females of any one culture carry identical wild second chromosomes, wild flies hatching in the F_3 cultures are homozygous for all genes on that chromosome. If the chromosome being tested carries no viability modifiers, the ratio of CyL/+ to +/+ flies is approximately 2: 1; if it carries a gene which decreases the viability, the frequency of wild flies is lowered. As a measure of standard viability of wild type flies we have chosen 31.8 per cent which is the average frequency of wild flies in 561 cultures containing non-lethal chromosomes from population No. 3. Lethals are defined as chromosomes which permit fewer than 10 per cent of the expected number of homozygous flies to survive.

The results of the samples which have been analyzed to date are given in table I and in figure 1.

One obvious change occurring in these populations is the increase in the frequency of lethals in the control population. If these lethals have little effect in

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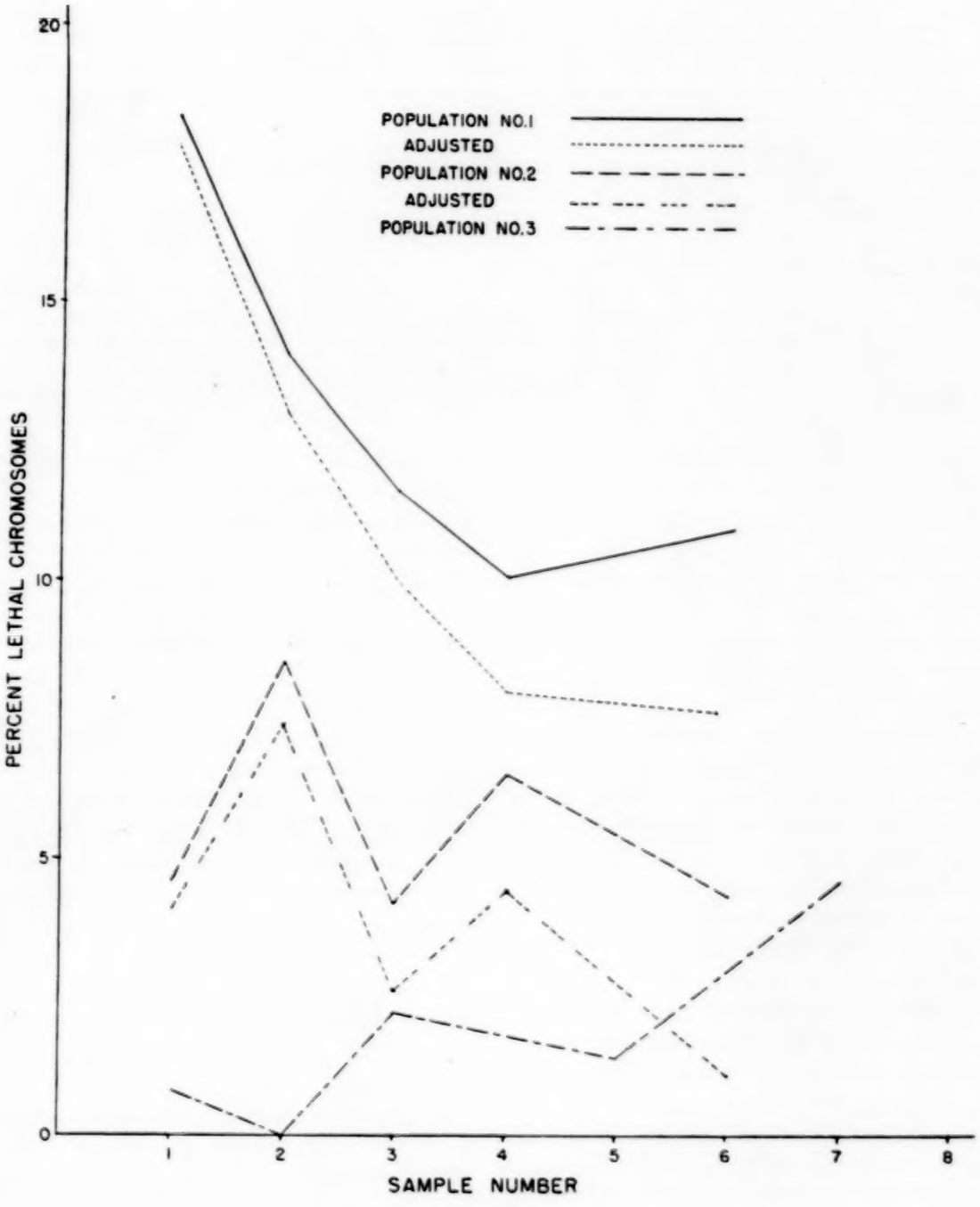

FIG. 1.

heterozygous individuals, their frequency at any time during the early generations should be the product of the mutation rate times the number of generations which have elapsed since the start of the population. The mutation rate which best fits our data is 0.53 per cent lethals per generation, a figure which is in agreement with values obtained by other workers (Dubinin, 1946). No prediction can be made from these data as to the ultimate frequency of lethals in the control population but it may be noted that after seven generations this frequency compares favorably with that induced originally in population No. 2.

Another obvious change is that which has occurred in population No. 1; the

TABLE I. *Frequencies of lethals in experimental populations.* n = number of chromosomes tested. Figures in parentheses represent frequencies adjusted as explained in text.

Sample	Population No. 1		Population No. 2		Population No. 3	
	n	freq.	n	freq.	n	freq.
1	131	18.3 (17.8)	152	4.6 (4.1)	133	0.8
2	156	14.1 (13.0)	164	8.5 (7.4)	52	0
3	173	11.6 (10.0)	168	4.2 (2.6)	183	2.2
4	178	10.1 (8.0)	186	6.5 (4.4)	—	—
5	—	— —	—	— —	212	1.4
6	202	10.9 (7.7)	188	4.3 (1.1)	—	—
7	—	— —	—	— —	261*	4.6*

* Tentative.

frequency of observed lethals has dropped from 18 per cent to 10 per cent in three generations. (Chi-square test for homogeneity gives $p = .2$, chi-square test combined with rank correlation gives $p = .05$–$.10$, chi-square sample 1 versus sample 4 gives $p = .03$). This decrease is almost certainly the result of semi-dominant effects of these induced genetic variants on the reproductive capacity of heterozygous individuals. Since these data are obtained from a breeding population, this lowered reproductive capacity may reflect a decrease in viability, a reduction of fertility (e.g., by certain chromosomal rearrangements), or both.

By assuming that spontaneous lethals accumulate in populations No. 1 and 2 at the same rate as in the control population, we may adjust the frequencies of lethals observed in those populations and reveal the changes which have occurred in the frequencies of induced lethals. These adjusted frequencies are shown in figure 1. Except for sampling error there is no reason to expect the frequency of induced lethals to increase; indeed, this frequency should remain constant if (or, when) only recessive lethals are present. (The small decrease in the frequency of recessive lethals resulting from the elimination of homozygotes would be very difficult to demonstrate experimentally.)

It is obvious that the frequency of induced lethals has decreased in population No. 1 (rank correlation of the 5 adjusted points gives $p < .01$). The present data are insufficient, however, to show that the decrease in the frequency of induced lethals in population No. 2 is real or that the small decrease in the frequency of induced lethals in population No. 1 (samples 4–6) represents the stabilization which should follow the elimination of incompletely recessive lethal genes.

We may conclude that the evaluation of the lasting genetic effects of an exposure of a population to acute radiations is not to be obtained from mutation rate data alone. The reproductive capacities (in the sense of contributions to a common gene pool) of individuals heterozygous for induced mutations must be determined in order to estimate the proportion of the original induced mutations which persist in the population and become part of its store of genetic variability. The data presented here indicate that less than one-half of the original induced lethals have persisted through six generations; the fate of the other half remains to be seen.

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NOTES AND COMMENTS

THE SPECIES CONCEPT: FURTHER DISCUSSION

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Recent discussions of this topic by Dr. Burma and Dr. Mayr are thought-provoking and each, I believe, is partly right.

Dr. Mayr is certainly justified in maintaining that each species in the living world is a natural biologic unit. Except for rare instances in which speciation is still hardly complete, none is capable of interbreeding with other species, even with sympatric relatives. Isolation *in space*, whether geographic or edaphic, has permitted small increments of change to build up to a point where gene-flow is broken, and thereafter the species stands apart as an objective reality.

But unless evolution has proceeded by saltations of specific or superspecific value, no species was ever isolated *in time*, either from its forbears or from its descendants, since the smallest break in gene-flow would have caused extinction.

Suppose then that we could bring back to life representatives of every generation of animals that ever existed. What problems would they pose for the systematists? Obviously the tree of life would be altered in no respect except that some of its lower branches could be shown in more detail and placed with greater confidence. But we should now have living populations at every level up and down every branch of the tree; and from any point in any branch, gene-flow, both up and down, will be not only possible but inevitable (for of course any generation of animals will interbreed with its immediate ancestors and its direct descendants). The flow will, of course, be along the branch to its junction with another branch and then either up or down, and so on ad infinitum. Gene-flow will thus be possible from the very base of the tree of life to its highest tip. How then shall we be able to draw a specific boundary to separate the lowliest protozan at the base of the tree from Dr. Mayr at the tip of its highest branch!

Dr. Burma thus has a point when he argues that the animal kingdom is divisible into phyla and classes and orders and genera, but not into species. For of course the fantasy indulged in above is no fantasy at all if we include in our reckoning the fourth dimension, time; those animals *did live* and genes *did flow* without a break, from the most primitive to the most specialized types of life, and the paleontologist has to deal with them all. To be sure, gene-flow has been essentially upward to the unborn, as ancestors died away; and it was never reversible as postulated above; but the flow was never broken.

This might suggest that the species concept of the paleoentologist must ever remain fundamentally different from that of the neontologist. Actually the situation is not so frustrating as it seems at first thought. The paleontologist deals only with fragments of the record. Fossiliferous rocks now available were formed in shallow seas, or in basins on the land, in either case so limited geographically as to afford merely samples of the life of the world at any given time. Even in these areas no sequence of strata is known in which the record of life is preserved unbroken for even a small part of geologic time. Diastems that appear only as bedding planes may denote non-deposition for hundreds or even thousands of years; and disconformities, both large and small, record still greater breaks. In most formations, furthermore, species appear suddenly as immigrants from regions unknown and migrate away or die out as the environment changes. Actually the paleontologist deals with tiny bits and pieces of the tree of life, all locked in their proper sequence in the rocks, but isolated one from the next because of lost segments. From the systematist's point of view the imperfection of the paleontologic record is a boon, because in this way each genetic line is effectively broken into species *isolated in time*.

The species of the neontologist may then be likened to a microtome slice out of a tiny twig on the tree of life; it has breadth but no appreciable depth. The species of the paleontologist, on the contrary, is a segment of such a twig, having both breadth (geographic distribution) and depth (in time). Specific characters vary in the former only in the horizontal plane, and in the latter in both directions. Even so, variations in time are likely to be similar in degree and in kind to those that occur in space, for they both represent the normal gene change in a continuously interbreeding population. Therefore unless the segments known to the paleontologist have considerable length, his conception of the species will not differ from that of the neontologist enough to cause difficulty or confusion. Even though the paleontologist can never apply interbreeding as a criterion of specific limits, this, too, should seldom cause trouble since, as Dr. Mayr has pointed out, the layman and even primitive peoples can recognize the distinctness of most of the species accepted by systematists.

Occasionally, however, the paleontologist encounters a fossiliferous sequence of rocks in which deposition was nearly continuous over a considerable span of time and in which the

fossil record is abundant and relatively complete. He then faces a very real and practical difficulty for it is impossible to draw satisfactory specific limits. A fine illustration may be seen in the Jurassic ammonites of the genus *Cosmoceras* studied by Roland Brinkmann about Petersboro, England. Here he collected over 3,000 specimens of this genus from 1,300 distinct zones in a thickness of about forty-three feet of shale. The specimens from each zone were then studied biostatistically. Four genetic lines (subgenera) could be followed through, and in each of these there were progressive but gradual changes in such obvious characters as the number of ribs, bundling of ribs into nodes, the development of spines, and the growth of lateral crests at the aperture. In each genetic line shells from the lower zones differ greatly from those in the uppermost zones—so greatly that if seen alone they would unhesitatingly be assigned to different species. Indeed in subgenera *Kosmoceras* s.s. and *Zugokosmoceras*, the change is so great that Brinkmann recognized, in each, five named species ranging one above the other. But since each grades insensibly into the next he distinguished for each "species" an early and a late subspecies and then placed between the late subspecies of one and the early subspecies of the next a "transitional" subspecies. The subgenus is thus assigned fourteen named subspecies each grading completely into the next above and below. Admittedly this is a completely artificial and arbitrary device in order to distinguish units for purposes of description and analysis.

This is perhaps an extreme case, but it affords a fine illustration of the practical difficulties a paleontologist may encounter in applying the species concept. Carruthers' classical study of the evolution of the coral, *Zaphrentis delanouei*, in the Lower Carboniferous rocks of Great Britain is a comparable case. Other instances somewhat less extreme are by no means uncommon in certain parts of the record. Delimitation of brachiopod species in the Pennsylvanian system in the Mid-Continent region, for example, is troublesome and unsatisfactory for anyone who has extensive collections from many horizons. A good many "species" range throughout a thickness of 2,000 to 3,000 feet of strata; specimens from the higher horizons commonly differ appreciably from those in the lower; but to decide where in a graded series to draw a specific boundary is a vexing if not insoluble problem. This region was part of a great interior seaway in which evolution of an autochthonous fauna continued with only minor interruptions during Pennsylvanian time. The underlying Mississippian system, on the contrary, includes a number of stratigraphic subdivisions each of which is characterized by highly distinctive faunas, clearly migrants from outside this area. As I well know from personal experience, it is as easy to distinguish brachiopod faunal zones in the Mississippian rocks of this region as it is difficult in the Pennsylvanian system.

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PALEONTOLOGIC VERSUS NEONTOLOGIC SPECIES AND GENERA

Maxim K. Elias

The recent chain of scientific events in America, which resulted in bringing closer together neontologists and paleontologists upon the ground common to both, such as systematics and evolution, resulted also in collision of their current concepts of such elementary scientific terms as species, genus, mutation. No matter what may be the eventual outcome of this collision, no doubt can possibly exist as to its immediate value for both groups of scientists, since through it they are getting mutually educated, and their minds are broadening.

Because of the natural temperamental differences in the scientific personalities involved in the collision, the attitudes of men on either side of the impact (and particularly on paleontological side of it) may be roughly classified into two categories: in one are those (eager-beavers) who are willing to trade readily their formerly held concepts to those used by their contemporaries in the opposite group; and in the other category are those (doubting Thomases) who feel that no such rush to trade the concepts is desirable or necessary, as they believe that the concepts of the terms when used in neontology and paleontology are naturally different because

of the inherent differences that exist between the two realms in which the two sciences (neontology and paleontology) operate.

We may be thankful to paleontologist Benjamin Burma for a provoking discussion of the Species Concept which drew sparks from neontologist Ernst Mayr in a form of an immediate reply and which thus brought to the fore the nature of this inherent difference. This is the addition of fourth dimension, which is *time* in Paleontology.

As Mayr in his "Semantic versus semantic" reply clearly states, the *"non-dimensional species* (Mayr, 1946) is the standard of the species concept as originally conceived by Ray and Linneus. The objective reality of *this* species is beyond doubt." And so indeed it is, and with this statement Burma (Postscriptum) has no quarrel, and neither have I.

However, to me the same holds true also for neontologic versus paleontologic genera, that is genera too have objective reality in neontology, but not necessarily in paleontology. In an address on the "State of Paleontology" which was read in March of 1949 (Jour. Pal., vol. 24, No. 2, March 1950) I endeavored to demonstrate with several examples that genera as we paleontologists define and use them never appear suddenly, but go through a phyletic state of inception, frequently lasting for an appreciable stretch of geologic time; and in many cases in turn are phyletically transformed into succeeding genera, also through a geologically perceptible period of instability or inception. Hence paleontologic genera frequently cannot be delimited objectively from those stratigraphically below or above them, unless, of course, their time range is being cut by extinction. Therefore the situation with paleontologic genera is much the same as with paleontologic species, because the denial of objective reality to the ubiquitous paleontologic species is based likewise on the impossibility of objective delimitation of one species from another, when the latter is developed from it and succeeds it in geologic time. Thus for the paleontologist both genus and species are mere temporal cross-sections of an endless continuity of changes, no segment of which, cut from above and below (in the course of geologic time), can possibly constitute an objective reality.

It may be added that the conclusion with regard to paleontologic genera is based not on theoretical speculation (whose usefulness I do not deny) but on prolonged investigation of several groups of late Paleozoic invertebrates. It was surprising for me to find, in the course of this work, that I was tempted to continue to use some specific names onward in some lineages where certain characters have changes in a sense that is generally agreed to be of generic significance. The surprising thing was that at the same time the characters, which are customarily regarded as of specific value, were found to continue in the lineage as if the supposedly more important generic changes never happened. Thus I have found that in the contemporary *paleontologic practice* we have some species which transcend, as it were, paleogeneric boundaries; and I have found that it is also our practice to give them new specific names whenever they cross such boundaries. This practice is obviously arbitrary, being based on a tacit assumption (but not an observation) that whenever the generic constitution of a species has changed, its specific constitution must have changed as well, even if this is not evident for our perception.

Now in making obvious these "secrets," so to speak, of our paleontological "trade," I may be branded as a traitor to paleontology, as I thus expose them for criticism by neontologists. They may suggest, for instance, that here is where we have gone astray, making genera on characters that should not be considered of generic importance. Perhaps in some cases it is so, just as this may be also the case in some neontological genera—but it is easier to say so than to attempt a radical revision of the paleontological genera now in use. What seems more important, however, is the realization that paleontologic species *and genera* differ profoundly from species and genera with which neontologists are dealing. It may be important, for instance, to keep this in mind when working toward a revision of the existing Rules of Zoological Nomenclature.

Finally, it seems important to observe that paleontologists, too, may be obliged to resort to neontological concepts of taxonomic terms, which would be the case when they are working on exceptionally prolific fauna or faunas from a *single geological horizon*, that is from a temporal cross-section of a group of lineages comparable to that of the present. Whenever such a single horizon fauna yields hundreds and even thousands of well preserved specimens of certain fossil organisms, we may attempt to recognize in these all genera, species, subspecies, clones, etc. in the neontological sense. But here too the situation becomes again peculiarly paleontologic as soon as we begin to compare the taxonomic units in such horizons with those from similar horizons stratigraphically above or below. It is exactly the situation with which Dr. Burma now finds himself confronted when studying the prolific group of fusulines classified in the comprehensive genus *Triticites*, and it is this realistic problem of handling the species in it that influenced to a considerable extent his discussion on the Species Concept in Paleontology.

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